UNIVERSITY OF CALGARY

The Effect of Exercise on Cardiac Metabolism in the Diabetic Heart

by

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The undersigned certify they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled *"The Effect of Exercise on Cardiac Metabolism in the Diabetic Heart"* submitted by Karen D. Ross in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

Diabetes is associated with an increased risk of cardiomyopathy, which is further exacerbated by physical inactivity. The oxidation of palmitate (μmol/min/g dw) and glycogen content including total glycogen, proglycogen and macroglycogen (μmol/g dw) was measured after 6 weeks of exercise training in *ex vivo* perfused working hearts from 12-week old diabetic mice, using both type 1 diabetic (T1D; streptozotocin-induced insulin deficiency) and type 2 (T2D) *db/db* models of diabetes.

Exercise significantly decreased the profound hyperglycemia that characterized T1D and T2D mice, but further enhanced diabetes-induced myocardial glycogen content. Exercise did not alter cardiac fatty acid oxidation rates. Exercise did not improve contractile function in diabetic hearts.

No studies have been done on the effects of exercise on metabolism and cardiac function as assessed using *ex vivo* working heart perfusions from diabetic mice. These results are novel suggesting exercise training may not be beneficial but rather detrimental to the diabetic heart, possibly perpetuating lipotoxicity.

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DEDICATION

I would like to dedicate this work to my family; with your generous financial support, love, great patience and encouragement this accomplishment was made possible. From the bottom of my heart, thank you David and Pen, San, Cameron, and Lucille and John. To dear Andrea and Mark, thank you for your never-ending belief in your Mom and to my husband Wayne, my biggest fan who has never stopped believing.

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LIST OF ABBREVIATIONS

ACC	-	Acetyl CoA Carboxylase
ALB	-	Albumin
AMP	-	Amplifier
AMPK	-	AMP- activated protein kinase
APO	-	Apolipoprotein
ACS	-	Acyl CoA Synthetases
ATP	-	Adenosine Triphosphate
BSA	-	Bovine Serum Albumin
CO	-	Cardiac Output
CHD	-	Coronary Heart Disease
CM	-	Chylomicron
CPT	-	Carnitine palmitoyltransferase
CVD	-	Cardiovascular Disease
Da	-	Dalton
EDTA	-	Ethylenediamine Tetraacetic Acid
ETC	-	Electron Transport Chain
EX	-	Exercise
FA	-	Fatty Acid
FABP	-	Fatty Acid Binding Protein
FAT	-	Fatty Acid Translocase
FATP	-	Fatty Acid Transport Protein
G 1-P	-	Glucose 1-Phosphate
GLUT	-	Glucose transporter
GN-1	-	Glycogenin-1
GO	-	Glucose Oxidase
HDL	-	High Density Lipoprotein
HR	-	Heart Rate
HSL	-	Hormone Sensitive Lipase
IRS	-	Insulin Receptor Substrate

KHB	-	Krebs Henseleit Bicarbonate Buffer
LA	-	Left Atrium
LPL	-	Lipoprotein Lipase
LCAD	-	Long Chain acyl-CoA Dehydrogenase
LPR	-	Lipoprotein Receptor
LV	-	Left Ventricle
MG	-	Macroglycogen
MCD	-	Malonyl CoA Decarboxylase
MCAD	-	Medium Chain acyl-CoA Dehydrogenase
NEFA	-	Non-Esterified Fatty Acid
Mr	-	Relative molecular weight
PCA	-	Perchloric Acid
PDH	-	Pyruvate Dehydrogenase
PSP	-	Peak Systolic Pressure
PG	-	Proglycogen
PI3K	-	Phosphatidylinositol-3-Kinase
PKB	-	Protein Kinase B
PPAR	-	Peroxisome-Proliferator-Activated Receptor
RPP	-	Rate Pressure Product
SED	-	Sedentary
STZ	-	Streptozotocin
SV	-	Stroke Volume
T1D	-	Type 1 Diabetes
T2D	-	Type 2 Diabetes
TG	-	Triacylglycerol
UCPs	-	Uncoupling Proteins
UDP	-	Uridine Diphosphate
UTP	-	Uridine Triphosphate
VLDL	-	Very Low Density Lipoprotein

CHAPTER ONE: INTRODUCTION.

1. Diabetes mellitus

Diabetes is a disorder of metabolic dysregulation where cells are not able to metabolize carbohydrates due to defects in insulin secretion, insulin action, or both (Shulman, 2000). However, diabetes is as much a disorder of fatty acid (FA) metabolism as it is a disorder of glucose metabolism (Young et al., 2002a). In this setting of altered metabolism, the body is exposed to an environment of lipotoxicity and glucotoxicity producing long-term damage and dysfunction: microvascular complications and failure of various organs, especially the eyes (retinopathy), kidneys (nephropathy), nerves (neuropathy), heart (coronary heart disease and cardiomyopathy) and blood vessels (endothelial dysfunction) (Saltiel, 2001). There are two main forms of diabetes, type 1 and type 2. Type 1 diabetes is characterized by hypoinsulinemia, hyperglycemia and hyperlipidemia, whereas type 2 (insulin-resistant) diabetes mellitus is characterized by initial hyperinsulinemia, hyperglycemia, and hyperlipidemia (Young, McNulty, & Taegtmeyer, 2002b).

1.1 Type 1 diabetes

The etiology of type 1 diabetes is characterized by a lack of insulin, a situation created by cellular-mediated autoimmune destruction of the β-cells in the islets of Langerhans of the pancreas (Grundy et al., 1999). Since type 1 diabetes is associated with eventual absolute insulin deficiency, hormone replacement therapy with insulin is required to avoid diabetic ketoacidosis and other diabetic complications which are associated with significant morbidity and mortality. It is not known why this autoimmune

diabetes develops, however it is associated with a complex interaction between genes and environment. There is a genetic tendency (Cudworth, Wolf, Gorsuch, & Festenstein, 1979) and sometimes type 1 diabetes follows a viral infection such as mumps, rubella, cytomegalovirus, measles, influenza, encephalitis, polio or Epstein-Barr virus (Dahlquist, 1995). Type 1 diabetes usually begins early in life (juvenile diabetes) with the greatest increase in the incidence of type 1 diabetes mainly occurring in children aged less than 5 years (Karvonen et al., 2000). The predominant risk factor for coronary heart disease (CHD) in patients with type 1 diabetes is duration of disease (Grundy et al., 1999). This type 1 form accounts for about 10% of all cases of diabetes.

1.2 Type 2 diabetes

Type 2 diabetes is the most common type of diabetes, accounting for 90% of all diagnosed cases. A strong genetic basis exists for type 2 diabetes as approximately 90% of patients with type 2 diabetes have a positive family history of this disorder (Stumvoll, Goldstein, & van Haeften, 2005). Not only is there a heightened genetic susceptibility of certain familial/ethnic groups for diabetes, insulin resistance develops as a result of obesity, particularly central or visceral obesity, physical inactivity, and a high fat diet. Therefore, type 2 diabetes is perhaps as much a disease of modern lifestyle as it is a disease of genetic susceptibility (Zimmet, Alberti, & Shaw, 2001). Type 2 diabetes is usually diagnosed after the age of 30, although the age of onset is progressively decreasing and now develops in children and adolescents as well as in young adults (Zimmet et al., 2001; Bloomgarden, 2004). Insulin resistance is recognized as an early and characteristic trait of type 2 diabetes and is defined as a state of reduced responsiveness by insulin sensitive tissues to normal circulating levels of insulin (Saltiel,

2000). It involves metabolic defects in liver and peripheral target tissues, such as adipose and muscle tissue and pancreatic β-cells, which all contrive together to produce abnormal glucose and lipid metabolism. Development of type 2 diabetes follows a timeline where resistance to the actions of insulin precedes the onset of defects in insulin secretion (beta-cell secretory dysfunction) that ultimately produce hyperglycemia and the type 2 phenotype (Cavaghan, Ehrmann, & Polonsky, 2000; Saltiel, 2001).

1.3 Prevalence

Type 2 diabetes has become a worldwide epidemic infiltrating not only our westernized societies but also developing countries. Africa, Asia and South America are predicted to see the greatest increases in the prevalence of type 2 diabetes over the next two decades (Zimmet et al., 2001). The total numbers of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (Wild, Roglic, Green, Sicree, & King, 2004). In 2002, at least 1.2 – 1.4 million Canadians aged 12 or greater carried a diagnosis of diabetes mellitus, and another 35 – 44% were estimated to have undiagnosed diabetes (Dawson, Gomes, Gerstein, Blanchard, & Kahler, 2002).

The global economic and disease burden associated with diabetes is large and continues to grow (King, Aubert, & Herman, 1998; Zimmet, 2003; Yach, Stuckler, & Brownell, 2006). The total Canadian economic cost of diabetes and its chronic complications in 1998 was between $4.76 and $5.23 billion (in U.S. dollars) (Dawson et al., 2002). In those people just with diagnosed diabetes, the direct medical costs associated with diabetes care, before considering any complications, were $573 million. Of the costs associated with the complications of diabetes, cardiovascular disease was by far the greatest, at $637 million (Dawson et al., 2002). Diabetes is associated with severe

morbidity and mortality when not treated and places a prodigious burden on health care resources, making it a prime target for prevention.

2. Diabetes is associated with increased risk of cardiovascular disease (CVD)

Cardiovascular disease (CVD) is a major cause of death in diabetic patients (Kannel, Hjortland, & Castelli, 1974); furthermore, diabetes is now considered an independent risk factor for CVD in both men and women. Diabetic women may even lose their cardioprotective status against cardiovascular events (Grundy et al., 1999; Kannel et al., 1974). The risk of myocardial infarction in an individual with diabetes but without evidence of overt heart disease is the same as for a nondiabetic patient who has already had a myocardial infarction (Haffner, Lehto, Ronnemaa, Pyorala, & Laakso, 1998). Thus, once a person with diabetes develops clinical evidence of CVD, their likelihood of survival is worse than that of nondiabetics with cardiovascular disease (Haffner et al., 1998).

Nearly 25 years ago, the post-mortem findings and clinical records of 27 patients with diabetes showed left ventricular hypertrophy in the absence of major coronary artery disease (Rubler et al., 1972). Until then the increased cardiovascular morbidity and mortality of diabetic patients was attributed to vascular disease, namely atherosclerosis in the coronary vasculature. Now the cardiac complications associated with diabetes have been revised. There are two recognized pathophysiological processes. First, coronary heart (ischemic) disease is increased as a consequence of accelerated atherosclerosis due to conventional and novel risk factors such as abdominal obesity, hypertension, dyslipidemia (elevated triglycerides, reduced HDL, and the presence of small dense LDL)

and pro-thrombotic factors such as fibrinogen and plasminogen activator inhibitor (PAI-1) which promote systemic inflammation (Hayden & Reaven, 2000; Kreisberg, 1998). Second, a unique diabetic cardiomyopathy exists (Hayat, Patel, Khattar, & Malik, 2004; Rubler et al., 1972; Fang, Prins, & Marwick, 2004).

2.1 Diabetic cardiomyopathy

Diabetic cardiomyopathy is a problem of the heart muscle and is defined as ventricular dysfunction in the absence of coronary heart disease or hypertension (Hayat et al., 2004). Diabetic cardiomyopathy is characterized by diastolic dysfunction and its etiology is related to insulin resistance (Phillips et al., 1998). Cardiomyopathy follows a developmental timeline. Insulin resistance initiates changes at the cellular level causing myocardial abnormalities that culminate in left ventricular hypertrophy and diastolic dysfunction. This is followed by altered contractile performance (systolic dysfunction) (Hayat et al., 2004).

Characteristic of diastolic dysfunction is a defect in active relaxation, and also a passive stiffness of the left ventricle caused by interstitial fibrosis demonstrated without clinical signs of heart failure (Bertoni, Tsai, Kasper, & Brancati, 2003). Changes in diastolic function are a widely reported finding in diabetic animals (Semeniuk, Kryski, & Severson, 2002; Mihm, Seifert, Coyle, & Bauer, 2001) and diabetic patients without evidence of heart disease caused by other factors (Giampietro et al., 1997; Raev, 1994; Zarich, Arbuckle, Cohen, Roberts, & Nesto, 1988). Systolic dysfunction is impairment in the ability of the heart to eject blood, and occurs later in the context of diabetic cardiomyopathy, often when patients have already developed significant diastolic

dysfunction. Animal, epidemiological and clinical studies have all shown diabetes to be associated with systolic dysfunction (Joffe et al., 1999).

3. Hormonal control of blood glucose and fatty acid concentrations

To carry out normal metabolism, cells need a continuous supply of glucose, one of the major fuels for producing cellular energy (ATP). Homeostatic blood glucose levels are normally maintained around 4-7 mM (Saltiel & Kahn, 2001). Fluctuations in blood sugar concentration regulate the release of two important but opposing hormones from the pancreas, insulin and glucagon. Rising glucose levels (most obvious just after a meal has been eaten) stimulate insulin-producing cells, the β-cells of the islets of Langerhans in the pancreas, which respond by secreting insulin into the blood. Insulin action targets both skeletal and cardiac muscle, hepatocytes and adipocytes. Insulin inhibits the production and release of glucose by the liver by inhibiting gluconeogenesis and glycogenolysis. Therefore, insulin suppresses hepatic glucose output and promotes peripheral glucose disposal.

While insulin is important in the utilization of glucose, glucagon is responsible for glucose output from the liver in response to low blood glucose levels (during fasting). Glucagon is secreted from pancreatic α-cells and produces its effects on intracellular pathways by binding to receptors in the cell membrane of hepatocytes (Frayn, 2003), to accelerate glycogenolysis and gluconeogenesis and thus increase hepatic glucose output.

Glucose enters cells by carrier-mediated diffusion using tissue-specific glucose transporters. There are 12 structurally related members of the glucose transporter family, called GLUT-n where n represents a number distinguishing different members (Bell,

1995). GLUT-1 is primarily found in the heart, fetal tissue, erythrocytes, placenta and brain blood vessels. GLUT-2 is predominately found in liver, kidney, intestine and pancreatic β-cells. GLUT-3 is responsible for glucose transport in neuronal tissue. GLUT-4 mediates insulin-stimulated glucose uptake by skeletal muscle, heart, and white and brown adipose tissue. GLUT-5 is the glucose transporter found mainly in the intestine (Bell, Burant, Takeda, & Gould, 1993). The effect of insulin, exercise (muscle contraction) and ischemia increase glucose uptake, largely by intracellular GLUT-4 recruitment (translocation) to the cell surface (Gao, Ren, Gulve, & Holloszy, 1994; Russell, III et al., 1998). Conversely, the diabetic state, decreasing workload and the presence of fatty acids as an alternate substrate reduce glucose uptake (Randle, Newsholme, & Garland, 1964).

Insulin also regulates FA metabolism. Under fed (post-prandial) conditions, insulin promotes hepatic lipogenesis (FA synthesis from glucose) and the production and secretion of triacylglycerol-rich very-low-density lipoproteins (VLDL). Circulating VLDL together with chylomicron particles containing dietary triacylglycerols (TG) are degraded by lipoprotein lipase (LPL), an enzyme located on the endothelial surface in adipose tissue, heart and skeletal muscle. Adipose tissue LPL activity is enhanced by insulin, therefore calories derived from either carbohydrates or lipids in the diet are preferentially stored in adipose tissue.

Another prime target for insulin is to inhibit lipolysis in adipose tissue, preventing the release of fatty acids by inhibiting the enzyme hormone sensitive lipase. Therefore, insulin lack (fasting or in diabetes) results in hyperlipidemia. FA concentrations are elevated in blood because lipolysis is no longer restrained by insulin; TG-rich lipoprotein

concentrations in blood are elevated due to both increased production and decreased catabolism of VLDL.

4. Metabolic abnormalities in the diabetic heart contribute to contractile dysfunction

Effective cardiac contraction requires synthesizing just the right amount of ATP to match the rate of ATP hydrolysis to ensure this chemical energy fuels cardiac contractile work and relaxation. Not only does ATP provide energy for contractile work, but ATP is required for pumping calcium into the sarcoplasmic reticulum to allow for diastolic relaxation, and for maintaining ion gradients (Na^+ and K^+). In fact, one-third of the ATP hydrolyzed is used for ion pumps (Stanley & Chandler, 2002). In the heart, about 60 – 70% of ATP generation in the mitochondria comes from beta-oxidation of fatty acids, and 10-40% comes from pyruvate (formed from lactate and glycolysis) oxidation (Neely, Rovetto, & Oram, 1972). Perturbation of myocardial energy metabolism will affect mechanical efficiency and contractile function.

In diabetes, carbohydrate use decreases so that fatty acid oxidation can provide from 90 – 100% of the heart's ATP requirements (Lopaschuk, 1996). This altered metabolism stems basically from a defect in the action of insulin (Kantor, Lopaschuk, & Opie, 2001). Insulin plays a role in many diverse processes in carbohydrate and lipid metabolism. Insulin affects all areas of glucose metabolism mainly by controlling the transport of glucose; insulin also directly affects intracellular glucose disposal including glycogen and triacylglycerol synthesis, glycolysis and glucose oxidation. As well, insulin increases cell permeability to fatty acids (FA) by inducing fat transporters to the plasma

membrane (Luiken et al., 2002). In addition, insulin will suppress gluconeogenesis, lipolysis and protein degradation (Frayn, 2003). Thus, insulin dysregulation in diabetes produces defects in the action of insulin which results in acute metabolic derangements in both fuel supply and utilization by heart tissue. The etiology of diabetic cardiomyopathy is complex and obviously a number of factors over a chronic period of time are potential mechanisms in its pathology (Rodrigues, Cam, & McNeill, 1998).

4.1 Carbohydrate metabolism in the diabetic heart

Insulin increases glucose transport into cardiomyocytes by activating translocation of GLUT-4 from an intracellular vesicular compartment to the plasma membrane (Goodyear & Kahn, 1998). Increased glucose transport increases glucose utilization, therefore, insulin affects all areas of cardiac glucose metabolism by controlling the transport of glucose (Kantor et al., 2001). Processes which are dependent upon insulin will be reduced in the insulin-deficient or insulin-resistant diabetic state (Kantor et al., 2001). The rate-limiting restriction for glucose utilization in the diabetic heart is the slow rate of glucose transport across the sarcolemmal membrane into the myocardium, which probably results from the cellular depletion of glucose transporter GLUT-4 (Rodrigues et al., 1998). Furthermore, any defect in any component of the signal transmission pathway between the insulin receptor and GLUT-4 translocation could lead to insulin resistance of the type associated with type 2 diabetes. Glycolysis is depressed in the diabetic cardiomyocyte as a result of a reduction in the transport and uptake of glucose into cells (Randle, Priestman, Mistry, & Halsall, 1994a). The impaired glucose oxidation in the diabetic heart can result from a decreased rate of glucose phosphorylation likely initiated by elevated fatty acids as a result of insulin resistance. The classic Randle

cycle (Randle, Garland, Hales, & Newsholme, 1963) proposed high levels of FA can stimulate the tricarboxylic acid (TCA) cycle and increase citrate levels, which in turn inhibits phosphofructokinase in the glycolytic pathway, thereby reducing the rate of glycolysis leading to decreased glucose oxidation (Rodrigues et al., 1998). Therefore, it appears that reciprocal regulation of fatty acid and carbohydrate oxidation (Randle cycle) plays a role in mediating the pathological effects of high fatty acid concentrations in diabetes (Belke, Larsen, Lopaschuk, & Severson, 1999). In addition, another explanation for the reduced oxidation of glucose by the diabetic heart is that elevated FA oxidation increases the acetyl CoA to CoA ratio which activates the pyruvate dehydrogenase kinase to phosphorylate and inactivate the pyruvate dehydrogenase complex (PDH) (Randle et al., 1994a).

In diabetes, accelerated rates of fatty acid utilization impede glucose oxidation and favor glycogen synthesis (Neely & Morgan, 1974). Once substrate flow through the glycolytic pathway is inhibited there is a build-up of glucose 6-phosphate in the cardiomyocyte which activates glycogen synthesis and inhibits phosphorylase (Chen and Ianuzzo, 1982). As a result, glucose entering the cardiomyocyte is stored as glycogen that accumulates in huge quantities and interferes with key signalling pathways involved in glucose utilization (Laughlin, Petit, Jr., Shulman, & Barrett, 1990; Higuchi, Miyagi, Nakasone, & Sakanashi, 1995; Nakao, Matsubara, & Sakamoto, 1993; Conlee & Tipton, 1977). The altered activity of the glycogen synthase and phosphorylase systems as well as decreased activity of phosphofructokinase may be responsible for the elevated storage of myocardial glycogen (Chen & Ianuzzo, 1982).

The steps in glycogen synthesis are shown in Figure 1. Briefly, UDP-glucose is added to apo-glycogenin, an auto-glycosylation protein that initiates glycogen granule formation (Lomako, Lomako, & Whelan, 1988). Glycogenin is a substrate, catalyst and enzyme of glycogen granule formation, with 7-11 glucose residues added to a single tyrosine residue in the protein (Alonso, Lomako, Lomako, & Whelan, 1995). Two isoforms of glycogenin are differentially expressed in cells and tissues; glycogenin-1 (GN-1) is the predominant form in heart (Mu, Skurat, & Roach, 1997). Following glycosylation, GN-1 acts as a substrate for proglycogen synthase which together with branching enzyme forms a glycogen granule called proglycogen (Figure 1). In proglycogen (Mr 400 kDa), the glycogen granule has grown in tiers around the GN-1 core. The addition of more glucose residues results in a larger mature glycogen granule (Mr 10^7) termed macroglycogen (Figure 1). Proglycogen and macroglycogen exist in a dynamic equilibrium. Although it is well known that glycogen content is elevated in diabetic hearts (Laughlin et al., 1990; Higuchi et al., 1995; Nakao et al., 1993; Conlee & Tipton, 1977), the role of glycogenin and the proportion of total glycogen in proglycogen and macroglycogen have not been investigated.

The cellular role of glycogen has expanded beyond that of a simple storage form for glucose. In addition to this metabolic role, glycogen can be considered as a dynamic organelle-type structure with a number of associated enzymes, the "glycosome" (Shearer & Graham, 2004). Thus, glycogen can have a regulatory function as a scaffolding structure. Finally, glycogen can influence insulin sensitivity; glycogen accumulation in muscle can produce insulin resistance (Jensen et al., 2006). Consequently, it is important

to understand the chemical nature of glycogen in diabetic hearts and discover the implications of glycogen accumulation in relation to cardiac function.

4.2 Fatty acid metabolism in the diabetic heart

The increase in fatty acid metabolism in the diabetic heart is likely the result of many contributing factors. Insulin deficiency/resistance removes the normal insulin constraint on adipose tissue lipolysis, so plasma FA concentration increases (Shulman, 2000). In the diabetic heart, the acute increase in plasma FA delivery results in enhanced FA utilization with decreased glucose utilization, cardiac effects of the Randle cycle. The chronic over-supply of FA to the diabetic heart results in the activation of peroxisome proliferator-activated receptor (PPARα), part of the nuclear-receptor superfamily responsible for regulating the transcription of genes responsible for lipid and glucose metabolism (Simonson & Kendall, 2005). Activation of PPARα via naturally occurring ligands including FA, increases the expression of genes involved in three major steps in the cellular FA utilization pathway (Figure 2): 1) fatty acid transport (enhanced expression of FATP, FAT/CD36, FABP) and activation by fatty acyl CoA synthetase (ACS); 2) FA mitochondrial import by increased expression of carnitine palmitoyltransferase (CPT-1) and indirectly by increased expression of malonyl-CoA decarboxylase (MCD) which will reduce the inhibitory effect of malonyl-CoA on CPT-1; and 3) mitochondrial and peroxisomal oxidation (e.g., medium and long-chain acyl-CoA dehydrogenases (MCAD and LCAD), and uncoupling protein-3 (UCP3) (Barger & Kelly, 2000). As PPARα activation induces the expression of PPAR regulated genes (Figure 2), there is a concomitant increased fatty acid utilization by the diabetic heart (Gilde et al., 2003; Finck & Kelly, 2002; Sharma et al., 2004). Inhibition of pyruvate dehydrogenase

(due to the combined effects of PDK4 induction and FA-derived acetyl-CoA) limits pyruvate oxidation to further exacerbate glucose utilization (Young et al., 2002b). The impact of an enhanced cardiac FA metabolism in the diabetic heart has negative consequences in terms of cardiac function (Figure 3), namely production of a diabetic cardiomyopathy (Carley & Severson, 2005). One consequence is the development of lipotoxicity because the cell tends to store excess FA when the rate of oxidation is less than the rate of FA uptake (Schaffer, 2003). Intracellular triacylglycerol accumulation may induce apoptosis of the cell through the production of de novo ceramide formation (Zhou et al., 2000).The obese ZDF rat has shown cardiomyopathy secondary to cardiomyocyte lipid accumulation (Zhou et al., 2000). In addition, enhanced FA oxidation creates a mitochondrial source of reactive oxygen species (ROS) (Listenberger, Ory, & Schaffer, 2001). Finally, enhanced FA utilization by diabetic hearts can have deleterious effects on contractile function by reducing cardiac efficiency (Figure 3).

4.3 Experimental animal models allow assessment of direct deleterious effects of a diabetic cardiomyopathy

Many of the early studies used to elucidate the direct deleterious effects of diabetes on cardiac function (diabetic cardiomyopathy) without concomitant atherosclerosis came from type 1 diabetic rats. When streptozotocin (*stz*, an antibiotic extracted from *Streptomyces achromogenes*) was injected into the animals, their pancreatic β-cells were selectively destroyed (necrosis); inflammatory islet lesions indicated a cell-mediated immune reaction had taken place (Like & Rossini, 1976; Tomlinson, Gardiner, Hebden, & Bennett, 1992). These *stz*-injected type 1 diabetic animals resulted in a well-characterized model of insulin deficiency and hyperglycemia

(Tomlinson et al., 1992). Convincing evidence from biochemical changes in the heart were able to demonstrate that diabetes produces a decrease in cardiac function in isolated hearts and cardiomyocytes (Vadlamudi RV, Rodgers, & McNeill, 1982; Jackson, McGrath, Tahiliani, Vadlamudi, & McNeill, 1985; Lopaschuk, Katz, & McNeill, 1983). Alterations in glucose metabolism (Lopaschuk, 2002) contributing to contractile dysfunction in *stz*-induced diabetes mellitus (Tomlinson et al., 1992) were attributed to high levels of circulating FA and alterations in the control of FA oxidation (Lopaschuk, 2002) and have been linked to a decrease in GLUT-4 protein and mRNA levels (Neely & Morgan, 1974; Fang et al., 2004; Camps et al., 1992).

Experimental evidence for diabetic cardiomyopathy quickly exploded into the research milieu with the addition of mouse models of type 1 and type 2 diabetes to the repertoire of experimental animals. The cardiovascular consequences of type 1 diabetic mice, including chemically *stz*-induced diabetic mice and a genetic model of type 1 diabetes, the nonobese diabetic (NOD) mouse, have shown decreased cardiac function (Severson, 2004). Both systolic and diastolic dysfunction was revealed by *in vivo* and *ex vivo* analyses.

Rodent models of type 2 diabetes include the *ob/ob* mouse which is leptin deficient (Leibel, Chung, & Chua, 1997; Coleman, 1978). The *ob/ob* model shows hyperphagia, obesity and insulin resistance. Two rat models with the same leptin receptor mutation, the Zucker fatty rat (*fa/fa*) and the Zucker diabetic rat (ZDF), both develop hyperglycemia (but to different degrees) and both show obesity and insulin resistance (Sparks et al., 1998; Phillips et al., 1996). Diabetic C57BL/KsJ- $lepr^{db}$/ $lepr^{db}$ (*db/db*) mice are a monogenic model of type 2 diabetes, characterized by a point mutation on

chromosome 4 resulting in a leptin receptor mutation (Leibel et al., 1997). The leptin receptor ($Lepr^{db}$) mutation produces an obese type 2 phenotype with hyperinsulinemia and hyperglycemia when the mouse is homozygous recessive for the mutant leptin receptor gene. The lean heterozygote control (*db*/+) carries a single copy of the mutant leptin receptor and one normal copy of the leptin receptor gene, and is phenotypically normal with respect to body weight and plasma concentration of glucose, lipids and insulin. There are measurable increases in plasma insulin levels as early as day 10 in *db/db* mice, with beta cell hyperplasia and hypertrophy (Coleman, 1978). FA oxidation is elevated at 6 weeks in *ex vivo* perfused working hearts from *db/db* mice. Glucose oxidation is normal at 6 weeks when *db/db* hearts are compared to *db*/+ controls, but becomes reduced to 27% of control at 12 weeks of age (Aasum, Hafstad, Severson, & Larsen, 2003). Signs of cardiac mechanical dysfunction are evident at 10-12 weeks. Thus, diabetes-induced metabolic changes and contractile dysfunction have been reported in the *db/db* mouse model (Belke, Larsen, Gibbs, & Severson, 2000); diabetic *db/db* hearts exhibit signs of progressive cardiomyopathy (Aasum et al., 2003).

The cardiac consequences of type 2 diabetes with obesity and insulin resistance are similar to results obtained from studies with *stz*-induced type 1 diabetes, including a decrease in contractile performance, altered metabolism and an increase in susceptibility to ischemia-reperfusion injury (Severson, 2004).

5. Physical inactivity: an independent risk factor for diabetes-induced CVD

Sedentary habits and low cardiorespiratory fitness exacerbates insulin resistance, which finally culminates in a progression from normal glucose and lipid metabolism to

type 2 diabetes (Kannel et al., 1974; Bassuk & Manson, 2005; Katzmarzyk et al., 2003). It is generally accepted that a major factor in the increasing prevalence of diabetes with associated CVD is low levels of activity-related energy expenditure (Bassuk & Manson, 2005; Albright et al., 2000). Doing less physical labour, becoming more obese, and consuming food in greater amounts and with a much higher percentage of fat all conspire to increase the incidence of diabetes worldwide. Lifestyle changes accompanying technological advances have largely factored physical activity out of daily life (Booth, Gordon, Carlson, & Hamilton, 2000). For example, a 1997 physical inactivity Canadian profile demonstrated that 62% of Canadians are not active enough to derive health benefits from their physical activity (Canadian Society for Exercise Physiology, 2003). Similarly, only 34% of Canadians aged 25 to 55 are meeting the exercise recommendations in Canada's *Physical Activity Guide to Healthy Active Living*, which calls for an hour of low-intensity physical activity every day, or 30 to 60 minutes of moderate-intensity physical activity or 20 – 30 minutes of vigorous intensity physical activity four to seven days per week (Canadian Society for Exercise Physiology, 2003). In a 1996 Canadian survey it was reported that 36% of the deaths from all causes among adults were due to the main diseases associated with physical inactivity: coronary heart disease, stroke, colon cancer, breast cancer and type 2 diabetes (Katzmarzyk, Gledhill, & Shephard, 2000). Sedentary habits and low cardiorespiratory fitness are independent predicators of cardiovascular events and premature mortality in individuals with diagnosed diabetes (Albright et al., 2004; Grundy et al., 2002). Given the convincing scientific evidence that physical inactivity leads to diabetes and associated CVD and

premature death, the promotion of a physically active lifestyle is an important public health objective.

6. Effect of exercise on the diabetic heart

Prevention of insulin resistance and treatment of its main characteristics are now considered of utmost importance in order to combat the epidemic of type 2 diabetes mellitus and to reduce the increased risk of cardiovascular disease and all-cause mortality (Chipkin, Klugh, & Chasan-Taber, 2001). Regular physical activity is considered a cornerstone for non-pharmacological treatment in the management of type 2 diabetes mellitus (Li, Culver, & Ren, 2003; McGavock et al., 2004). Exercise can retard the progression of diabetes from one stage to another (Albright et al., 2000).

6.1 Effect of regular exercise on the heart

Healthy persons as well as many patients with CVD and diabetes can improve their exercise performance with training. Research has shown that physical exercise of sufficient intensity and duration improves cardiac performance and cardiac reserve in healthy non-diabetic individuals (Hiatt, 1991). The impact of exercise on cardiac function in diabetes displays somewhat similar patterns although patients with mild hyperglycemia show a better response than those patients with severe hyperglycemia. This improvement is derived from an increased ability to use oxygen to obtain energy for work. Exercise training increases maximal ventilatory oxygen consumption (VO_2max) by increasing the volume of blood ejected by the heart per minute (cardiac output) and improving the ability of the exercising muscles and heart cells to extract oxygen from blood (Morris & Froelicher, 1991; Morris & Froelicher, 1993). Other exercise training benefits result in

decreased myocardial oxygen demands for the same level of external work performed. These changes are important in maintaining quality of life, especially for patients who have had a cardiovascular event such as myocardial infarction or angina. After exercise training these individuals may attain a higher level of physical work before reaching the level of myocardial oxygen requirement that results in myocardial ischemia and consequently fatigue (Clausen & Trap-Jensen, 1976). Exercise training favourably alters blood glucose levels, lipid metabolism, and reduces insulin resistance (Li et al., 2003). To receive the traditional training-related changes in cardiorespiratory fitness, the exercise must be sufficient and regular to sustain this benefit since prolonged training will bring about longer-lasting structural changes in the heart associated with an increase in heart rate (HR), stroke volume (SV) and/or myocardial contractility (Li et al., 2003).

6.2 Beneficial effects of exercise on cardiac function in human diabetes

The possible benefits of exercise in type 2 diabetes are substantial. Research has shown that routine exercise regimes reduce blood glucose, blood pressure, body weight and body fat, and improve lipid profiles (Li et al., 2003). Initially exercise guidelines referred mainly to glycemic control, whereas the recognition of the benefits of exercise training on diabetic cardiovascular health is now becoming more obvious (Stewart, 2002). For example, the Nurse's Health Study examined the relationship between levels of physical activity and incidence of coronary heart disease and stroke among women with type 2 diabetes. Hu et al (1999) concluded that diabetic women who increased their physical activity by spending at least 4 hours per week performing moderate or vigorous exercise had an approximately 40% lower risk for cardiovascular disease compared to those that did not exercise. In another study among a diverse group of adults with

diabetes, walking was associated with a 39% lower all cause mortality and a 34% lower cardiovascular disease mortality. It was estimated that one death a year could be prevented for every 61 people who would walk at least two hours a week (Gregg, Gerzoff, Caspersen, Williamson, & Narayan, 2003). In the Health Professional's follow-up study (HPFS) (Tanasescu, Leitzmann, Rimm, & Hu, 2003), physical activity was associated with reduced risk of CVD, cardiovascular death, and total mortality in men with type 2 diabetes after 14 years of follow up. Epidemiological data further suggest that high cardiorespiratory fitness in men with type 2 diabetes is associated with better prognosis and may reduce the risk of CVD associated with the disease (Wei, Gibbons, Kampert, Nichaman, & Blair, 2000). Interestingly, compared with structured aerobic exercise, moderately intense activity has demonstrated similar benefits on cardiorespiratory fitness. For example, equivalent energy expenditure in walking and vigorous exercise results in similar risk reduction in coronary heart disease (Manson et al., 2002) and type 2 diabetes (Hu et al., 1999) in the main cohort of the Nurses Health study.

6.3 Beneficial effects of exercise on cardiac function in animal models of diabetes

Several type 1 diabetic animal studies have shown that exercise training improves myocardial function (Broderick, Poirier, & Gillis, 2004; Hall, Sexton, & Stanley, 1995; Osborn, Daar, Laddaga, Romano, & Paulson, 1997; Paulson, Kopp, Peace, & Tow, 1987; De Angelis et al., 2000). As noted above, insulin resistance will decrease GLUT-4 content in the sarcolemma by interfering with the translocation of GLUT-4 transporters to the plasma membrane from intracellular vesicles (Goodyear & Kahn, 1998). In *stz*-induced type 1 rats moderate intensity exercise has been shown to reverse this trend and

restore basal rates of glucose uptake (Hall et al., 1995). In another study, diabetes was induced in male Sprague-Dawley rats by intravenous injection *of stz* (50 mg/kg) through the tail vein (Paulson et al., 1987). Animals were trained on a treadmill for 60 minutes at 27m/min, 6 days per week for 8 weeks. Cardiac output and work were measured in isolated working hearts perfused at various preload pressures and with buffer containing physiological concentrations of glucose and FA. Training of diabetic rats improved cardiac pump function (Paulson et al., 1987). In another study, Broderick et al. (2004) demonstrated that exercise training attenuated the decrease in heart rate and aortic flow found in sedentary type 1 diabetic rats, with aortic flow normalized to control hearts. Not only did exercise training improve cardiac function, but exercise training increased tolerance to ischemia in the type 1 diabetic rat heart, possibly because rates of glucose oxidation were higher in hearts from exercised diabetic rats (Broderick et al., 2004). After a period of low-flow ischemia, rat hearts recovered function during reperfusion; both heart rate and aortic flow were improved compared to sedentary diabetic hearts. These exercise-induced changes in cardiac function are further supported by studies examining ultrastructural changes in type 1 diabetic rat hearts. Nine week old male Sprague-Dawley rats were administered a single injection of *stz* (65 mg/kg) after a two week exercise training acclimatization period. Exercise was found to significantly attenuate diabetes-induced changes in collagen fibrils, cytoplasmic area, and level of mitochondrial disruption (Searls, Smirnova, Fegley, & Stehno-Bittel, 2004).

Studies with type 2 diabetes have shown exercise will produce insulin sensitization (Hawley & Houmard, 2004; Hansen & Wojtaszewski, 2002) and improve glucose disposal into skeletal muscle (Perseghin et al, 1996). Insulin-stimulated glucose

uptake into cardiomyocytes from Zucker rats was enhanced after exercise (Huisamen & Lochner, 2005); an increase in cardiac protein kinase B (PKB) phosphorylation after exercise has been demonstrated in Zucker rats (Huisamen & Lochner, 2005) and ZDF rats (Lajoie et al., 2004). Exercise improved contractility in rat hearts with diet (sucrose)-induced insulin resistance (Davidoff et al, 2004). However, there are at this point relatively few investigations into the cardiac consequences of exercise with type 2 animal models. No studies on effects of exercise on cardiac metabolism and cardiac function have been assessed using *ex vivo* perfusions with beating hearts from type 2 diabetic rodents.

6.4 Achieving metabolic fitness with low-intensity endurance exercise

Traditionally, the emphasis of training was aimed at increasing VO_2max through moderate- to high-intensity exercise regimens of 60% - 85% VO_2max (Chipkin et al., 2001). However, a goal of treatment in type 2 diabetes should be one of metabolic fitness emphasising an important treatment role for 'therapeutic lifestyle change' rather than in improvements associated with traditional measures of cardiorespiratory fitness (increased VO_2max, decreased heart rate and increased cardiac output) (Despres & Lamarche, 1994). Prolonged endurance exercise of low intensity, performed on an almost daily basis, significantly improves metabolic variables considered as risk factors for diabetes through mechanisms that are likely independent from training-related changes in cardiorespiratory fitness (Romijn et al., 1993; Despres & Lamarche, 1994). Thus, metabolic fitness is achieved at lower exercise intensity. Further, endurance training at low – moderate intensities will induce metabolic adaptations that result in increased fat oxidation (Coyle, 1995).

6. Hypothesis

Exercise will attenuate diabetes-induced metabolic dysfunction and improve cardiac function measured with *ex vivo* perfused hearts from diabetic mice.

7. Statement of Objectives

This study is designed to evaluate cardiac function and metabolism, using *ex* vivo perfused hearts from both type 1 (*stz*-induced) and type 2 (*db/db*) exercised and unexercised diabetic mice.

1. To determine the effects of exercise on body weight and blood glucose concentrations after 6 weeks of aerobic training in type 1 and type 2 diabetic mice.
2. To assess the effects of exercise on cardiac fatty acid oxidation rates, using *ex vivo* working hearts from type 1 and type 2 diabetic mice.
3. To assess the effects of exercise on cardiac glycogen content in type 1 and type 2 diabetic hearts (perfused and non-perfused).
4. To assess the effects of exercise on contractile function of perfused hearts from type 1 and type 2 diabetic mice.

CHAPTER TWO: METHODOLOGIES.

1. Animal Models

All experiments were approved by the University of Calgary Health Sciences Animal Welfare Committee and followed the guidelines of the Canadian Council on Animal Care. All mice were housed in groups of four in the animal facility at the University of Calgary, maintained under a 12 hour light-dark cycle and given ad libitum access to food (standard laboratory chow) and water. Two different types of diabetic mice (type 1 and type 2) were used in this study.

1.1 Type 1 diabetic model:

Male CD-1 mice, age 5 – 6 weeks (n = 32), were acquired from the Jackson Laboratories (Bar harbour, ME). Type 1 diabetes was induced in half of the mice using an intraperitoneal injection of 215 mg/kg streptozotocin (*stz;* Sigma, St Louis, MO) dissolved in 0.10 M citrate buffer (pH 4.5, at a concentration of 30 mg/mL). *Stz* was administered to animals over a three day period (Kennedy & Zochodne, 2000). Control animals received only citrate buffer injections. On the first day, mice received 85 mg/kg *stz*, on the second day 75 mg/kg and on the third 55 mg/kg, for a total cumulative dose of 215 mg *stz* per kg mouse body weight. The duration of the *stz*-induced insulin-deficient type 1 diabetic condition was 1 week exercise protocol acclimatization plus 6 more weeks of training. Diabetes was confirmed through measurements of blood glucose levels, which were acquired by obtaining whole blood from each animal through a tail tip cut. Blood glucose was measured with a One Touch Ultra® blood glucose meter (Johnson and Johnson Company, Milpitas CA, USA). This method uses a whole blood sample which

is placed on a test strip. Reagents on the test strip contain an enzyme (glucose oxidase) that causes glucose to react with a dye to produce a color change. The color intensity is proportional to the amount of glucose present. The strip is placed in a small, hand-held meter that quantifies the glucose concentration using reflectance spectrometry. Results obtained by this method tend to have good agreement in regard to accuracy and precision with plasma glucose procedures obtained using a Beckman Glucose Analyzer II if done properly (Brunner et al., 1998; Poirier et al., 1998).

1.2 Type 2 diabetic model:

Diabetic C57BL/KsJ- $lepr^{db}$/ $lepr^{db}$ *(db/db)* mice were purchased from Jackson Laboratories (Bar Harbour, ME). Diabetic *db/db* mice express an early phenotype of insulin resistance, detectable at 10 days of age by hyperinsulinemia, with beta cell hyperplasia and hypertrophy (Coleman, 1978). By 12 weeks of age, *db/db* mice exhibit a severe type 2 diabetic phenotype, with marked obesity and profound hyperglycemia.

As with type 1 diabetic mice, diabetes in *db/db* mice was confirmed by acquiring whole blood from each animal by cutting the tip of the tail; blood glucose concentrations were measured with a One Touch Ultra® blood glucose meter (Johnson and Johnson Company, Milpitas CA, USA).

2. Exercise Training Protocol

Mice were randomly sorted into one of two groups: those participating in regular exercise, the diabetic EX (n = 8) group or those that remained sedentary, the diabetic SED (n=8) group. Control littermates followed the same regime, normal EX (n = 8) and normal SED (n=8) groups. The EX animals ran on a motorized wheel for 60 min per day,

5 days per week for 6 weeks. Workload was set to 8 seconds/rotation, thus each mouse ran a distance of 212 m/session and a total distance over 6 weeks of 6716 m. All EX mice were initially exposed to a low-intensity exercise as a method of acclimatizing the mice to the exercise protocol. This exercise protocol was adopted because of previous studies on the effect of exercise on endothelial function in mesenteric arteries from control and diabetic *db/db* mice (Dr. Andrew Howarth, Smooth Muscle Research Group, University of Calgary; personal communication).

Metabolic studies were conducted at 12-13 weeks of age, after completion of 6 weeks of exercise training. All trained mice were sacrificed 24 hours after their last workout session. 18 hours after the last bout of exercise is long enough for the acute effect of exercise on insulin responsiveness to wear off (Cartee et al., 1989).

3. Isolated perfused working mouse hearts

Studies have demonstrated that alterations in energy metabolism contribute to cardiac dysfunction, especially cardiomyopathies (Barr & Lopaschuk, 2000). It is therefore critical to have good experimental techniques to directly measure energy metabolism in the heart. The perfused working mouse heart is a good model for studying cardiac metabolism (Belke et al., 1999). It allows measurement not only of heart metabolism, but cardiac function simultaneously in *ex vivo* hearts without the influence of neurohumoral factors (Larsen et al., 1999). The perfusion apparatus allows for control of heart rate, the control of preload and afterload (work) and as well, the supply of substrate to the working cardiac cells.

Fatty acids (FA) are the preferred source of fuel for the heart; typically 60 – 70% of the heart's energy requirements come from FA metabolism (Saddik & Lopaschuk, 1991). The other major substrate of the aerobically perfused heart is glucose. It is easy to complicate measurement of energy substrate metabolism in the heart, for example, by not recognizing the glucose – fatty acid (Randle) cycle as a mechanism that integrates the utilization of FA and glucose. For instance, omission of FA from the perfusate dramatically increases the rates of myocardial carbohydrate metabolism (Saddik & Lopaschuk, 1991; Randle et al., 1963). Therefore it is important to measure cardiac energy metabolism by supplying hearts with relevant perfusate concentrations of both glucose and fatty acids (Barr & Lopaschuk, 2000). The method employed in this study measured fatty acid oxidation rates of palmitate bound to bovine serum albumin (BSA). Glucose was present in the perfusate but radioactive glucose was not utilized for concomitant determinations of glucose oxidation to simplify FA oxidation measurements.

3.1 Why a working heart?

It is desirable to use experimental models in which the heart is functioning at physiologically relevant workloads (Barr & Lopaschuk, 1997). The continuous contractile function of hearts and the high intrinsic metabolic rate inherent in hearts from small animals like mice are balanced. As a result, flux through glycolysis, glucose oxidation, lactate oxidation, fatty acid oxidation, tricarboxylic acid cycle activity, and electron transport chain activity is critically dependent on the work performed by the heart. Therefore, rates of ATP production by the heart depend on ATP utilization (Neely & Morgan, 1974). It is therefore desirable to ensure that the *ex* vivo perfused heart is functioning at physiologically relevant workloads during perfusion. This is achieved by

using an antegrade working heart perfusion technique (Figure 4), in which the left atrial pressure provides the preload or input to the heart. The left ventricle (LV) then ejects the perfusate into an aortic cannula, with afterload providing the outflow impedance against which the LV must eject (Barr & Lopaschuk, 1997). The preload is most commonly regulated by gravity-fed atrial filling, thus ensuring a constant pressure which is determined by the height of a column of buffer, the preload reservoir, and the size of the cannula. The left atrium is perfused at a constant preload pressure of 15 mmHg, while the LV pumps against a constant afterload pressure of 50 mmHg. The afterload is a column design as well, where a pressure head is created by a standing column of buffer with the meniscus situated 50 cm above the heart (Lopaschuk & Barr, 1997). Pressures are measured by a transducer placed in the aortic afterload line and calibrated with a manometer to the specified pressure (Figure 4).

3.2 Heart isolation and perfusion conditions

Mice received 100 U of heparin via intraperitoneal injection 20 minutes before they were anesthetized with a 10 mg intraperitoneal injection of sodium pentobarbitone. Heparin is used as an anti-coagulant to facilitate subsequent cannulations of the left atrium and aorta. The heart was excised and placed in chilled (4°C) Krebs-Henseleit bicarbonate (KHB) buffer solution, consisting of (in mM): 118.5 NaCl, 25 $NaHCO_3$, 4.7 KCL, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 2.5 $CaCl_2$, 0.5 EDTA and 11 mM glucose and is gassed with 95% O_2 and 5% CO_2 (pH 7.4) (Carley et al., 2004).

In the chilled KHB buffer, the aorta was cannulated with an 18-gauge plastic cannula (1.5 cm length; .095 mm, ID; 1.3 mm, OD) for Langendorff retrograde perfusion (60 mmHg perfusion pressure) with warm KHB buffer (Larsen et al., 1999) for

approximately 20 minutes to wash blood out of the heart (Belke et al., 2000). Hearts were stabilized in Langendorff mode, where perfusate is moving in a direction contrary to normal flow (retrograde perfusion), and held at a perfusion pressure of 60 mmHg. The perfusion buffer did not re-circulate as in working mode.

Following establishment of coronary perfusion in Langendorff mode, the left atrium (LA) was cannulated through the pulmonary vein with a 16-gauge steel cannula (1.14 mm, ID; 1.52 mm, OD) that was connected to the preload reservoir by a flexible tube (Larsen et al., 1999). Myocardial temperature during working mode was maintained close to 38°C since the tubing leading to the LA was water-jacketed and heated to 38°C. In addition, the LA preload reservoir (where the KHB buffer was oxygenated) and the buffer reservoir were also heated to 38°C. Once the heart was switched from Langendorff to working mode by closing the Langendorff line and opening the LA preload line, 35 mL of a modified perfusate was delivered to the LA and subsequently ejected (systole) from the LV through the cannulated aorta (Barr & Lopaschuk, 1997). The working heart buffer was a modified KHB buffer consisting of (in mM): 118.5 NaCl, 25 $NaHCO_3$, 4.7 KCL, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 2.5 $CaCl_2$, 0.5 EDTA and 0.4 mM [9,10-^{3}H] palmitate (specific activity = 0.43 μCi/μmolL) bound to 3% BSA and 11 mM glucose, gassed with 95% O_2 and 5% CO_2 (pH 7.4). The total fatty acid concentration was 0.7 mM, from 0.4 mM added endogenous FA plus 0.3 mM [9,10-^{3}H] palmitate bound to albumin (Carley et al., 2004). During diastole, oxygenated fatty acid-containing buffer is delivered to the coronary arteries of the heart. The LV ejects the perfusate through the aorta and into the afterload line, which houses a compliance chamber 16 cm upstream of the aortic cannula containing 2 mL of air, to act like a windkessel (mimicking the “elastic

recoil" of an artery wall) to drive blood, perfusate in this situation (Figure 4), through a closed system (Larsen et al., 1999). Physiologically relevant preload and afterload pressures were set at 15 mmHg and 50 mmHg, respectively. The modified perfusion solution re-circulated within a closed system, unlike the Langendorff preparation, so that the perfusion apparatus can be sealed.

Hearts were perfused for one hour with functional and pressure measurements occurring every 10 minutes. Each time the ventricle contracts and relaxes the coronary arteries are perfused. The coronary perfusate moves through the coronary circulation; eventually, the venous return empties into the right atrium and the perfusate exits from the pulmonary artery. The dripping pulmonary artery perfusate constitutes coronary flow. Coronary flow rates (mL/min) were measured by determining the time it took for 20 drops (1 mL) to drip from the bottom of the heart (Neitzel, Carley, & Severson, 2003). Aortic flow rates were measured by a timed collection from the flow in the afterload line (Belke et al., 2000). The sum of coronary and aortic flows represents cardiac output. As flow measurements were recorded, heart contractility (peak systolic pressure) and heart rate were measured with a pressure transducer in the aortic after-load line (Millar Micro-tip, Millar Instruments). Aortic pressure traces were recorded with CV Works software (University of Calgary, Calgary, Alberta, Canada).

Every 20 minutes, a 2.0 mL perfusate buffer sample was removed through an injection port for metabolic measurement (^3H_2O content; see below). The perfusate samples were stored in scintillation vials and stored at -80°C for future analysis. At the end of the perfusion period, the atria were trimmed, and hearts were frozen and stored at -80°C for the determination of ventricular dry weight. This was used to normalize

metabolic and flow data to correct for small variations in heart size (Carley et al., 2004). In addition to measuring dry weight, glycogen content of perfused hearts was determined (see below). Glycogen content of unperfused hearts was determined whereby hearts were quickly excised and placed in cold KHB buffer and subsequently rinsed to remove blood; atria were trimmed, and hearts were then frozen and stored at -80°C.

4. Measurement of palmitate oxidation

Two by-products of the fatty acid oxidation pathway are H_2O and CO_2. By labelling the fatty acids in the perfusate with ^{3}H, ^{3}H can be quantitatively collected and measured. By using radioisotopes that are labelled at specific hydrogen on the palmitate molecules, and by knowing the specific activity of the radiolabeled substrate used, it is possible to determine the actual rate of flux through this pathway. The rate of disappearance of radiolabeled palmitate (measured by the appearance of ^{3}H) is directly related to the rate of FA oxidation. How many palmitate molecules are removed (metabolized) per unit of time requires information about how much isotope there is per palmitate molecule in the pool. The amount of isotope per palmitate molecule is called the specific activity of the palmitate molecule.

Non-esterified FA are not water-soluble; they are carried in plasma bound to the plasma protein albumin. The working heart perfusate in this experiment contains a radioactive non-esterified FA, [9,10-^{3}H] palmitate bound to 3% BSA, thus ensuring transportability of FA to the working cardiomyocytes. To bind palmitate to BSA, radioactive palmitic acid was dissolved in 25 mL of water – ethanol mixture (15 mL water and 10 mL of ethanol) containing 0.55 g Na_2CO_3/g palmitate. The mixture was

boiled with constant stirring until all ethanol evaporated and then was poured quickly into the modified KHB solution containing 3% BSA (without glucose) while stirring vigorously to ensure adequate mixing. The solution was dialyzed (8000-12000 Mr cut-off; SPECTRAPOR, Spectrum medical Industries, LA, USA) in a 10x (vol) of KHB at 4°C overnight. The next morning glucose (11 mM) was added to the dialyzed solution and finally filtered (0.45µm, COSTAR, Fisher Scientific, Edmonton, Alberta, Canada) and frozen in 35 mL aliquots for future use.

The oxidation of [9,10-^{3}H]palmitate (0.7mM in the perfusate) was determined by measuring the release of ^3H_2O into perfusate samples collected at 20 minute intervals during the one hour working heart perfusion. Steady-state rates of fatty acid oxidation were determined by averaging the results from perfusate samples removed at the three time points (0-20, 20-40, 40-60 minutes) for each heart perfusion (Carley et al., 2004). ^3H_2O was separated from [9,10-^{3}H] palmitate by mixing 0.5 ml perfusate sample with 1.88 mL of chloroform:methanol (1:2) (vol/vol). This was followed by the addition of 0.625 mL of chloroform and mixing, and then 0.635 mL of 1.1 M KCl dissolved in 0.9 M HCl was added whereupon the samples were vortexed. Test tubes were centrifuged for 10 minutes at 10,000 rpm which results in separation of the polar and nonpolar phases. The bottom organic phase was discarded and the top layer, the aqueous phase, was subsequently treated with a mixture of 2 mL of chloroform:methanol (1:1 vol/vol), and 0.9 mL KCL:HCL (1.1 M/ 0.9 M), respectively. Test tubes were centrifuged for another 10 minutes at 10,000 rpm which again resulted in separation of the polar and nonpolar phases. Two 0.5 mL samples of the aqueous phase were then counted for each perfusate sample, for total ^3H_2O determinations, taking into account the dilution factor. Saddik &

Lopaschuck (1991) have shown that this technique results in a greater than 99.7 % extraction and separation of $^{3}H_2O$ from un-metabolized [9,10-^{3}H]palmitate. Calculation of palmitate oxidation rate using $^{3}H_2O$ was determined by the method outlined by (Barr & Lopaschuk, 1997). Palmitate oxidation rates are expressed as μMol/min/g dry weight.

5. Glycogen content

The method for determination of proglycogen (PG) and macroglycogen (MG) content (Figure 1) utilizes differential acid solubility; MG is acid-soluble due to its higher carbohydrate content whereas PG is acid-insoluble (Adamo & Graham, 1998; Adamo, Tarnopolsky, & Graham, 1998; Lomako, Lomako, & Whelan, 1991). Ice-cooled 1.5 M perchloric acid (PCA; 200 μL) was added to 20 – 30 mg of freeze-dried heart muscle samples in 5 mL pyrex tubes. The muscle was pressed against the glass tubes with a plastic rod to ensure that all of the muscle was exposed to acid. The extraction continued on ice for 20 minutes. The samples were centrifuged at 3,000 rpm for 15 minutes, after which 100 μL of the PCA supernatant was removed, placed in Pyrex tubes, and used for the determination of MG. The remaining PCA was discarded, and the pellet was kept for the determination of PG. 1 mL of 1 M HCl was added to the MG and PG samples; the MG sample was vortexed, whereas the PG pellet was pressed against the glass with a plastic rod. The tube weights were recorded and the tubes were then sealed with fitted glass stoppers (marbles); all of the samples were then placed in the water bath (100°C) for 2 hours, after which the tubes were reweighed. Any weight change greater than 50 μL was rectified with the addition of deionized water (assuming water loss). The samples were then neutralized with 2 M TRIS base since the following glucose assay is slowed

considerably at low pH, vortexed, centrifuged at 3,000 rpm for 5 minutes (Shearer et al., 2005). Samples were transferred to Eppendorf tubes for analysis of glucosyl units by using a Sigma Glucose (GO) Assay Kit (#GAGO-20). This is a glucose oxidase-peroxidase system for the enzymatic determination of glucose. Principally glucose was oxidized to gluconic acid and hydrogen peroxide by glucose oxidase. Hydrogen peroxide reacted with *o*-dianisidine, a chromogenic hydrogen donor, in the presence of peroxidase to form a colored product. Oxidized *o*-dianisidine further reacted with sulfuric acid to form a more stable colored product. The intensity of the final pink color measured at 540 nm was proportional to the original glucose concentration. The pink color was known to be stable for at least 12 hours (Washko & Rice, 1961). Glucosyl units were calculated (μmol/g dry weight) by first determining the amount of glucose (μg) in the assayed sample using the measured absorbance of the sample and the absorbance of the glucose standard (5 μg). This value was corrected with a dilution factor based on the total volumes of PCA, HCL and Tris used previously and divided by the assayed sample volume (100 μL), converted to μmol by dividing by the Mr of glucose and then divided by the dry heart weight for that sample.

6. Statistical analysis

Data is expressed as mean ± SEM. Differences in cardiac function and FA oxidation rates between exercised and unexercised non-diabetic and diabetic mice were analyzed using two-way ANOVA followed by Tukey statistical test for significance. Enzymatically determined glycogen was analyzed by two-way ANOVA followed by Tukey test. Differences between means were regarded as statistically significant at $p<.05$.

CHAPTER THREE: RESULTS.

1. Characterization of control and diabetic mice

This study primarily investigated the effects of exercise on cardiac metabolism (palmitate oxidation and glycogen levels), using hearts from both type 1 and type 2 diabetic (T1D and T2D) mice. Type 1 (insulin-deficient) diabetes was chemically induced in 5 – 6 week male CD1 mice with streptozotocin. Typically, streptozotocin-treated animals fail to gain weight and blood glucose levels are 3 to 4 times higher than normal (Tomlinson et al., 1992). Blood samples for glucose determination were collected from the tail tip and diabetes was confirmed prior to exercise training with T1D mice in this study.

Type 2 diabetic (*db/db*) mice have a mutation in the leptin receptor producing hyperphagia and obesity driven by the lack of leptin action (Unger, 2002). This *db/db* model displays early peripheral insulin resistance; hyperinsulinemia is a compensatory mechanism to initially allow normal blood glucose levels. Hyperglycemia finally develops when insulin secretion from beta cells can no longer keep up with peripheral insulin resistance. Obesity characterizes this model as body weights of *db/db* mice are usually double the weight of control mice (Hummel, Coleman, & Lane, 1972).

1.1 Body weights

In this study, CD1 and *db/+* mice were used as baseline controls to compare the effects of exercise training in T1D and T2D mice, respectively. Control *db/+* mice were significantly smaller than CD1 mice. Prior to initiation of exercise training, CD1 controls

weighed approximately 28 grams, while *db/+* mice weighted approximately 25 grams (Table 1). Throughout the training period, between 6 – 12 weeks of age, CD1 controls continued to exhibit significantly greater body weight than *db/+* mice (Figure 5). Exercise training did not significantly affect final mean body weight in either control group (Table 1, Figure 5). These results are consistent with other studies in mice (Niebauer et al., 1999; Lerman et al., 2002) and rats (Bowles, Farrar, & Starnes, 1992; Powers et al., 1993; Hall et al., 1995; Burelle et al., 2004). Conversely, there are several studies with rats that have demonstrated exercise training does significantly attenuate the increase in body weight exhibited by sedentary controls (Tancrede, Rousseau-Migneron, & Nadeau, 1982; Paulson et al., 1987; Osborn et al., 1997).

Type 1 (insulin-deficient) diabetes (T1D) was induced in CD1 mice using an intraperitoneal (i.p.) injection of 215 mg/kg streptozotocin (*stz*) at age 5-6 weeks. Mean body weight of sedentary (SED) T1D mice was significantly less than CD1 sedentary (SED) controls (19 ±1 vs 21 ±1 g respectively) at commencement of training, one week after *stz* administration (Table 1). Significant weight loss after induction of insulin-deficient type 1 diabetes is consistent with other studies in mice (George et al., 2002) and rats (Paulson et al., 1987; Hall et al., 1995; Osborn et al., 1997; Broderick et al., 2004). Mean body weight of sedentary (SED) and exercised (EX) T1D mice increased progressively to 31 ±1 g and 28 ±1 g respectively during the 6 week training program (Figure 6, Table 1). However, mean body weight continued to be significantly lower in T1D mice compared to controls throughout training (Figure 6). Exercise training did not significantly alter the final body weight in T1D mice (Table 1). These results are consistent with studies in T1D rats (Paulson et al., 1987; Broderick et al., 2004).

Non-diabetic db/+ control mice were considerably smaller prior to training compared to db/db mice (Table 1), consistent with previous results showing that *db/db* mice at six weeks of age already demonstrate increased body weight (Aasum et al., 2003). Mean body weight of sedentary (SED) and exercised (EX) db/db mice increased progressively to 49 ±2 g and 46 ±2 g respectively during the 6 week training program (Table 1, Figure 7). Exercise training did not significantly affect mean body weight in *db/db* mice. These results are consistent with studies in trained ZDF/*Gmi+/fa,* an insulin resistant animal model of type 2 diabetes (Lajoie et al., 2004).

1.2. Plasma glucose concentrations

Exercise training of *db/+* and CD1 control mice had no significant effect on blood glucose levels (Table 1). These results are supported by other studies in rats (Tancrede et al., 1982; Paulson et al., 1987; Houmard et al., 1991).

As expected, both untrained T1D and T2D SED mouse models demonstrated significantly higher levels of blood glucose (50 and 52 mM, respectively) compared to their non-diabetic controls (Table 1). Blood glucose levels decreased significantly in trained T1D mice compared to sedentary T1D mice (41 ±3 vs 50 ±2 mM). The beneficial effects of exercise training in lowering glucose levels in type 1 diabetic animals has been demonstrated in other studies (Osborn et al., 1997; Searls et al., 2004). Other observations reporting that glycemic control cannot be achieved with exercise alone in type 1 diabetic rats have also been reported (Woodiwiss, Kalk, & Norton, 1996; Tancrede et al., 1982; Paulson et al., 1987; Hall et al., 1995; Villanueva, Poirier, Standley, & Broderick, 2003).

Exercise training of type 2 diabetic mice significantly lowered plasma glucose levels (44 ±2 vs 52 ±1 mM) (Table 1). Similar results have observed that exercise enhanced the glucose lowering effects of metformin and acarbose drug treatments for type 2 diabetes in *db/db* mice (Tang & Reed, 2001) and lowered plasma glucose levels in insulin-resistant Zucker diabetic fatty (ZDF) rats (Lajoie et al., 2004).

2. Characteristics of hearts from control and diabetic mice

2.1. Heart weights

As noted above, *db/+* mice were smaller than CD1 mice. Heart dry weights from CD1 control mice weighed approximately 37 – 39 mg, while *db/+* mice had much smaller hearts, approximately 30 mg (Table 1).Values for dry heart weight were not significantly different for sedentary (SED) and exercised (EX) CD1 and *db/+* control groups. Therefore, six weeks of training did not induce myocardial hypertrophy in control mice (Table 1). These findings are consistent with other reports for rats (Paulson et al., 1987), however myocardial hypertrophy of individual cells due to training has been confirmed in cardiomyocytes isolated from left ventricles in both mice (Kemi, Loennechen, Wisloff, & Ellingsen, 2002; Evangelista, Brum, & Krieger, 2003; Allen et al., 2001; Kaplan et al., 1994) and rats (Burelle et al., 2004; Bowles et al., 1992; Powers et al., 1998).

Type 1 diabetic hearts also did not exhibit hypertrophy after training (Table 1). However in keeping with findings from other studies, T1D hearts were significantly smaller (~25 mg) compared to CD1 controls (Tomlinson et al., 1992). Training-induced cardiac hypertrophy was not evident in type 2 *db/db* hearts (Table 1).

2.2. Myocardial glycogen content and distribution

Content (μmol/g dry weight) of total myocardial glycogen (G_t), proglycogen (PG) and macroglycogen (MG) was determined after 60 minutes of normoxic perfusion for CD1 and *stz*-induced T1D hearts. In contrast, glycogen content in *db/+* and *db/db* hearts was assessed *before* and *after* one hour aerobic perfusion.

(a) Control CD1 and *db*/+ perfused hearts

When control hearts were compared, *db/+* SED perfused hearts had more PG and MG compared to CD1 perfused mouse hearts (Table 2). Comparative data for control (CD1 and *db/+*) hearts with (EX) and without (SED) exercise training is also shown in Figures 8 and 9. When the proglycogen content as a percent of total glycogen was calculated, PG made up approximately 35 – 40% of total glycogen content in CD1 SED and *db/+* SED control perfused hearts; these percentages were not significantly different between the two control SED groups (Table 2, Figure 8). Obviously, perfused *db/+* SED hearts had significantly higher total glycogen content compared to CD1 SED and CD1 EX hearts (Figure 9). Importantly, exercise did not have an effect on total glycogen content and its distribution between PG and MG for either mouse heart (Table 2, Figures 8 and 9).

(b) Control CD1 and *stz*-injected type 1 diabetic (T1D) hearts

Sedentary T1D hearts had more PG compared to control CD1 hearts (18 ±1 vs 11±2 umol/g) (Table 2). When PG content as a percent of total glycogen was calculated, the proportion of PG was significantly higher (59%) in T1D SED perfused hearts compared to CD1 control hearts (35%) (Table 2, Figure 10). Interestingly, total glycogen values were not significantly different between SED T1D and CD1 controls (Figure 11);

thus, the proportion of PG was increased in T1D SED hearts but total glycogen content was unchanged. An intriguing aspect of this data was the much higher total glycogen content in EX T1D perfused hearts compared to SED T1D perfused hearts (49 ±7 μmol/g vs 30 ±1 μmol/g) (Figure 11). This increase suggests there is a distinct effect of exercise on trained T1D hearts. Further, the higher total glycogen content in T1D EX hearts was characterized by both an elevated proglycogen (26 ±4 vs 17 ±1μmol/g) and an increased macroglycogen content (23 ±4 vs 12 ±1 μmol/g) compared to T1D SED (Figure 10). Proportionally, PG contributed 53% toward total glycogen content in T1D EX hearts compared to 34% in CD1 EX control hearts.

(c) Unperfused and perfused control *db/+* hearts

Total glycogen levels (7-8 μmol/g) were not different between sedentary and trained unperfused control (*db/+*) hearts (Table 3). Unexpectedly, there was a marked increase in total glycogen *after* perfusion in SED and EX control (*db/+*) hearts (43 ±4 and 48 ±3 μmol/g respectively), however, these totals were not significantly different between untrained and trained *db/+* hearts in either unperfused or perfused groups (Table 3). PG contributed about 50% toward total glycogen concentration in unperfused control (*db/+*) hearts (Table 3), irrespective of SED or EX status. PG as a percent of total glycogen decreased significantly after perfusion, when PG contributed 39-40% toward total glycogen in both untrained and trained *db/+* perfused hearts. Therefore, the elevated total glycogen after perfusion of *db/+* hearts was achieved by a 10% decrease in the proportion of PG and a concomitant 10% increase in MG.

(d) Unperfused and perfused type 2 diabetic (*db/db*) hearts

Unperfused T2D (*db/*db) SED hearts had significantly higher MG and calculated total glycogen levels but not PG (p=0.051) compared to *db/+* SED hearts (Table 3, Figures 12 and 13). Additionally, the PG:MG proportion was significantly different between unperfused *db/+* and *db/db* hearts. In both trained and untrained *db/db* hearts, PG contributed 36-37% toward total glycogen levels, whereas in control *db/+* hearts PG contributed about 50% toward the total (Figure 12). Unperfused EX T2D hearts were clearly responsive to the effect of exercise training as judged by significantly higher total glycogen levels in EX *db/db* hearts compared to unperfused *db/db* SED hearts (Figure 13). In fact, both PG and MG increased significantly compared to T2D (*db/db*) SED unperfused hearts (Figure 12).

Table 3 provides absolute values for PG, MG and total glycogen *after* perfusion of *db/+* and *db/db* hearts. After one hour perfusion with 11 mM glucose, there was a substantial significant increase in PG (Figure 14), MG (Figure 15) and total glycogen content (Figure 16) in T2D *db/db* hearts compared to unperfused *db/db* hearts. The PG percentage (Table 3) in perfused *db/db* SED hearts (37%) was unchanged compared to perfused *db/+* hearts (37%) and unperfused *db/db* hearts (37%). Thus, both *db/+* and *db/db* experienced net glycogen synthesis after perfusion. However, the increment in glycogen during perfusion of the *db/db* SED hearts clearly was much less than in control *db/+* hearts (Figures 14-16). In *db/db* EX hearts, perfusion resulted in a modest increase in MG (Figure 15) but not PG (Figure 14). Thus, in T2D *db/db* hearts, total glycogen concentration was no longer different after perfusion for both SED and EX *db/db* hearts

(28 ±2 vs 30 ±3 μmol/g; Figure 16), indicating that a differential effect of exercise was lost after perfusion.

(e) Comparison of type 1 and type 2 diabetic perfused hearts

Absolute values for PG, MG and total glycogen content of perfused T1D and T2D trained and untrained hearts demonstrated some striking differences between the two types of diabetes (Table 4). The most impressive difference was an elevated PG content in T1D hearts, especially in trained T1D EX hearts (Figure 17). Proglycogen levels were significantly greater in trained T1D EX hearts (26 ±4 umol/g) compared to either SED T1D hearts (18 ±1 umol/g) or T2D hearts (10 ±1 umol/g). MG was not significantly different between T1D SED and T2D hearts; however, MG was significantly elevated in T1D EX hearts compared to all other groups (Figure 17). Consequently, T1D EX hearts demonstrated an increase in total glycogen compared to total glycogen in T1D SED and T2D hearts (Figure 18).

2.3. Palmitate oxidation by perfused working hearts from trained and untrained control and diabetic mice

The isolated perfused working mouse heart provides an excellent model to examine the relationship between myocardial energy metabolism and physiological workload and energy demand. The present study is the first to assess the effect of training on fatty acid oxidation by *ex vivo* perfused working mouse hearts under normoxic perfusion conditions. It was hypothesized that exercise will attenuate the elevated fatty acid oxidation rates measured with *ex vivo* perfused hearts from diabetic mice.

Myocardial steady-state palmitate oxidation rates were not significantly different between trained *db/+* and CD1 hearts and their sedentary control littermates (Figures 19 and 20). Studies in control rats do not support these same findings. In fact, after prolonged training in control female Sprague-Dawley rats, a significant 50-65% *increase* in palmitate oxidation rate was observed (Burelle et al., 2004).

The oxidation of [9,10-^{3}H] palmitate by perfused hearts from sedentary T1D mice was significantly increased (0.72 μmol/g/min) relative to CD1 control littermates (0.40 μmol/min/g) (Figure 19). Previous evidence for the existence of elevated fatty acid oxidation rates in *stz*-induced type 1 diabetic hearts relative to control hearts have been reported (Tomlinson et al., 1992; Neitzel et al., 2003). There were no differences between sedentary and exercised *stz*-injected T1D hearts with respect to myocardial fatty acid oxidation (Figure 19); however the rate of palmitate oxidation in T1D EX hearts was no longer different from control CD1 EX hearts.

Palmitate oxidation rates were measured during a 60-minute aerobic working heart perfusion with *db/db* hearts. Previous studies have reported repeatedly that fatty acid oxidation rates are enhanced in type 2 diabetic hearts (Belke et al., 2000; Aasum et al., 2003; Hafstad, Solevag, Severson, Larsen, & Aasum, 2006; Carley et al., 2004). In contrast, this study did not observe elevated fatty acid oxidation rates in sedentary *db/db* hearts (0.18 ±0.01 μmol/g/min) relative to control *db/+* hearts (0.15 ±0.01 μmol/g/min). In addition, palmitate oxidation rates were not different between untrained and trained *db/db* hearts (Figure 20).

2.4. Cardiac function of ex vivo perfused working hearts

A number of contractile parameters were monitored at 10-minute intervals during the working heart perfusions. Heart rate (HR) and peak systolic pressure (PSP) was measured with a Millar pressure transducer inserted in the afterload line. Aortic and coronary flow rates were determined by timed collections of flow in the afterload line and the buffer dripping from the bottom of the heart, respectively (Belke et al., 2000). Aortic and coronary flows and cardiac output (CO) were calculated as mL per minute. Rate pressure product (RPP), the product of heart rate (HR) and peak systolic pressure (PSP), and hydraulic power, the product of CO and PSP, were also used to measure cardiac work.

Control *db/+* hearts showed no changes with exercise for any of the measured parameters of contractile function (Table 5). Trained CD1 control hearts showed no change in heart rate (Figure 21) or RPP (Figure 22) but unexpectedly exhibited a marked increase in cardiac power (Figure 23). The elevated hydraulic power in CD1 EX hearts was not attenuated when normalized for heart weight.

T1D EX hearts had a significantly lower heart rate (312±9 bpm) compared to T1D SED hearts (363 ±9 bpm) (Table 5, Figure 21); consequently, RPP was significantly lower in trained T1D hearts ($p=0.006$) (Figure 22). This same reduction in RPP was not evident in T2D hearts (Table 5, Figure 24). Mechanical performance was considerably more variable throughout the 60-minute perfusion period for *db/db* mice as denoted by the decline in RPP over perfusion time. Other than a significantly decreased RPP (HR * PSP) in T1D EX hearts, contractile dysfunction was not evident in either type 1 or type 2 diabetic hearts.

Aortic and coronary flows (ml/min) were not significantly reduced in diabetic hearts compared to controls (Table 5). Therefore, cardiac output (aortic flow plus coronary flow) was unchanged in all diabetic hearts. CO * PSP ($mmHg \cdot ml/min \cdot 10^{-3}$) was calculated over a 60-minute perfusion time at 10-minute intervals and did not show any significant differences in any of the diabetic experimental groups (Figures 23 and 25).

CHAPTER FOUR: DISCUSSION.

1. Model systems of choice

The Severson lab has routinely uses a chemically induced insulin-deficient type 1 diabetic mouse and a genetic model of type 2 diabetes, the *db/db* mouse, to study metabolic alterations that may be linked to contractile dysfunction in diabetic hearts using an *ex vivo* perfused working heart model (Severson 2004; Carley & Severson 2005). The mouse has now become the animal model of choice when studying changes in cardiac phenotype because mice can be genetically engineered to study some of the features of the diabetic cardiomyopathy. Studies have been conducted on mouse models that are not diabetic but have elevated levels of fat utilization, simulating a diabetic condition. For instance, hearts from transgenic cardiac-specific over-expression of peroxisome proliferator-activated receptor-α exhibits a metabolic phenotype very similar to diabetic hearts, with increased FA utilization (Finck et al., 2002). A mouse model with over-expressed long-chain acyl CoA synthetase (ACS) was developed to elevate the esterification of cellular fatty acids with coenzyme A and thus increase cardiac FA utilization (Chiu et al., 2001). Both of these transgenic mice were characterized by enhanced lipid utilization in hearts along with signs of contractile dysfunction. Genetically engineered mice with cardiac phenotypes resembling a diabetic cardiomyopathy with enhanced lipid utilization provide an opportunity to determine whether contractile function can be reduced in these nondiabetic states (Severson, 2004).

Limitations in the choice if animal models of diabetes used in this thesis research must be acknowledged. It is recognized that the STZ model represents severe

uncontrolled insulin deficient type 1 diabetes; in contrast, humans with insulin administration will typically have at least partial control of their blood glucose. Furthermore, the *db/db* type 2 animal model represents a severe monogenic phenotype, in contrast to human polygenic condition with interacting environmental factors.

The *ex vivo* perfused working heart model is a preferred model system to assess various contractile parameters including aortic flow, coronary flow and heart rate. The isolated perfused mouse heart allows measurements of cardiac function without influence of neurohumoral factors. With carefully controlled experimental conditions such as preload, afterload, heart rate, and supply of exogenous substrates, the isolated working heart allows measurement of cardiac metabolism and function simultaneously. The biggest challenge of an *ex vivo* methodology is achieving a stable working heart preparation given the small size (about 150 – 200 mg wet weight) and the high energy demand of the mouse heart. There is potential for damage to the heart during isolation and perfusion, and as well, because the mouse heart is so sensitive to ischemia-reperfusion damage, vigilant attention must be paid to the blood-free perfusate.

The Severson lab has reported reduced glucose utilization (decreased glycolysis and glucose oxidation), increased tissue levels of glycogen and increased fatty acid oxidation in *db/db* hearts (Belke et al., 2000; Aasum et al., 2002; Neitzel et al., 2003). Ultimately, the functional consequences of changes in cardiac metabolism may lead to contractile dysfunction in diabetic mouse hearts. Together fatty acid utilization (both oxidation and esterification) and glucose utilization (glycolysis and glucose oxidation) provide the most useful information about metabolism; however, FA oxidation is still a very useful parameter because it is altered in diabetic hearts. Aasum et al. (2003)

observed that an elevation in FA oxidation was the earliest metabolic change in perfused *db/db* hearts; at 6 weeks of age, FA oxidation was elevated when glycolysis and glucose oxidation were still unchanged.

2. Exercise as a therapeutic mechanism for targeting diabetic cardiomyopathy

Better understanding of the mechanisms behind metabolic disturbances in diabetic cardiomyopathy will be important in developing interventions to prevent or delay the development of diabetic cardiomyopathy and its complications. If the hypothesis that an altered diabetic metabolism can be a causative factor in cardiac contractile dysfunction is true, then an intervention designed to normalize the metabolism of diabetic hearts should also improve contractile function. The effectiveness of an exercise intervention directed at preventing and/or ameliorating endothelial dysfunction in *db/db* mice has been demonstrated (Dr. Andrew Howarth, Smooth Muscle Research Group, University of Calgary; personal communication). It was a natural progression, therefore, to examine the effectiveness of exercise as a therapeutic mechanism for targeting diabetic cardiomyopathy, using insulin-deficient type 1 and type 2 *db/db* mice to test the hypothesis that altered metabolism in diabetic hearts can be normalized with improvement of cardiac contractile function by regular endurance exercise training.

2.1 Exercise training and the dose-response relationship

A combination of frequency, intensity and duration of repeated bouts of exercise are responsible for producing a training effect. The interaction of these factors provides the required stimulus for myocardial adaptation which then responds with a new phenotype (Saltin et al., 1976). In general, if the stress or load is too small in intensity or

duration, little or no physical or metabolic adaptation is stimulated. Prolonged endurance exercise of low intensity, performed on an almost daily basis, significantly improves metabolic fitness (Despres & Lamarche, 1994).

Much of the improvement in certain health benefit indicators is achieved at lower volumes of physical exercise participation (e.g., reduction of triacylglycerol levels and blood pressure), while much of the improvement in other health benefit indicators comes at higher volumes of energy expenditure (e.g., increasing high-density lipoproteins) (Jakicic, Winters, Lang, & Wing, 1999). The total energy expenditure in physical activity has a dose-response relation to the effectiveness of health benefits (Jakicic et al., 1999). Research shows considerable health-benefits of moderate-intensity physical activity even in the absence of changes in the traditional measures of physical fitness status (Dunn et al., 1999). A dose-response relationship exists between amount of exercise and improvement in health benefit indicators.

The exercise intensity/protocol utilized in this study was a low-moderate intensity carried out 5 days per week for 1 hour per day and was one that had previously been shown to improve endothelial dysfunction in diabetic C57BL/KsJ- $lepr^{db}$/ $lepr^{db}$ *db/db* mice (personal communication from Dr. Andrew Howarth). Thus, the combination of frequency, intensity and duration of exercise was responsible for producing a training effect in *db/db* mice, at least in terms of vascular function. The interactions of these training parameters conspired to create a stimulus causing adaptation and a new phenotype, one where endothelial dysfunction was attenuated. This study too demonstrated that chronic exercise did in fact create a new phenotype in response to training. Whole-body glucose concentrations in both type 1 and type 2 diabetic mice

were lowered through the effects of training, and interesting alterations in cardiac glycogen stores were observed. However, this exercise training regimen did not change fatty acid oxidation rates or contractile function in type 1 and type 2 diabetic mice.

3. Effect of exercise on control and diabetic mice

In this study, CD1 and *db/+* mice were used as controls for eventual comparisons with the effects of exercise training in type 1 (insulin-deficient) diabetes (T1D) and type 2 (*db/db)* diabetes (T2D) respectively. Exercise had no effect on weight gain or final body weights for CD1 and *db/+* mice (Table 1, Figure 5), and produced no change in blood glucose (Table 1). Thus, investigating the effect of exercise on cardiac phenotype of diabetic mice was not confounded with a training effect on control mice.

Exercise training also had no effect on the weight gain of *stz*-treated CD1 (T1D) mice (Figure 6) or T2D (*db/db)* mice (Figure 7), or on final body weights (Table 1). However, exercise decreased the profound hyperglycemia that characterized these two diabetic models (Table 1). Blood glucose levels were 18% lower in trained T1D mice compared to sedentary T1D mice after 6 weeks exercise training. In addition, exercise training of type 2 (*db/db)* diabetic mice significantly lowered plasma glucose levels by 15%. Interestingly, the improvement in glucose homeostasis in *db/db* EX mice was not linked to any reduction in their obese phenotype. These improvements in whole body glucose homeostasis in T1D and T2D mice are likely due to effects on glucose transporters in heart and skeletal muscle and improved insulin sensitivity (Jessen & Goodyear, 2005; Holloszy, 2005).

The beneficial effects of exercise training in lowering whole body glucose levels in T1D and T2D animals has been demonstrated previously (Osborn et al., 1997; Tang &

Reed, 2001; Searls et al., 2004; Lajoie et al., 2004; Huisamen & Lochner, 2005) and appears to be an adaptive response to exercise training (Reaven & Chang, 1981). Moderate-intensity exercise training may confer improved insulin sensitivity, glycemic control and an improved metabolic profile.

Muscle glucose transport is dependent on the translocation of GLUT-4 transporters to the plasma membrane. GLUT-4 is the predominant isoform expressed in insulin-sensitive tissues such as the heart and skeletal muscle, and is responsive to insulin action and muscle contraction. Both mechanisms will activate molecular signalling pathways to induce GLUT-4 translocation, but the muscle contraction pathway is distinct and independent from the insulin-activated GLUT-4 translocation pathway (Goodyear & Kahn, 1998). The insulin activated GLUT-4 signaling pathway has been shown to be defective in diabetic humans and diabetic animals (Hall et al., 1995; Avogaro et al., 1990; Garvey, Hardin, Juhaszova, & Dominguez, 1993; Semeniuk et al., 2002; Penpargkul, Schaible, Yipintsoi, & Scheuer, 1980). Diabetes results in a substantial reduction in myocardial GLUT-4 expression and translocation due to insulin deficiency or resistance. Exercise training appears to acutely increase glucose transport for subsequent metabolism in muscle by recruitment of glucose transporter proteins. The diabetes-induced reduction in GLUT-4 was attenuated with exercise (Hall et al., 1995). From the results of this study, it can be concluded that exercise elicited improved glycemic control in both T1D and T2D mice, affirming other studies which have demonstrated that exercise increased muscle membrane glucose transport by contraction-induced glucose uptake.

4. Effect of exercise on control hearts from CD1 and *db/+* mice

Exercise had no effect on heart dry weights (Table 1), indicating that cardiac hypertrophy was not produced by this training protocol in control CD1 and *db/+* mice. Similarly, exercise did not change control heart metabolism, assessed by measurements of palmitate oxidation (Figures 19, 20) and glycogen determinations (Figures 8, 9, and 13). Total glycogen and glycogen distribution between PG and MG was unchanged in perfused control CD1 EX and *db/+* EX hearts compared to SED hearts (Table 2). In addition, exercise had no effect on glycogen content in unperfused *db/+* EX hearts or in the proportion of PG to MG as a percent of total glycogen (Figures 12, 13). Because of the high energy demand of beating mouse hearts under basal conditions, it is possible that GLUT-4 was already fully translocated to the cell membrane, to produce a transport maximum. Therefore, at this point no further exercise-induced increase in GLUT-4 translocation to the cardiomyocyte membrane can occur. Alternatively, the effect of exercise may still enhance GLUT-4 translocation to the cell membrane in control hearts with improved glucose uptake; however, glucose could be readily oxidized rather than stored as glycogen. Finally, contractile function was unchanged by exercise training (Table 5), except for CD1 EX hydraulic power, which is a by-product of an elevated cardiac output ($p=0.056$).

Significant health-related benefits of regular exercise are achieved at lower exercise intensity (but greater volume) than that required to improve performance/skill related fitness. Obviously, the stress or load of the exercise protocol used in this investigation was too small in either intensity or duration to stimulate cardiac adaptive

growth (physiological hypertrophy) in control mouse hearts. By comparison, a 10-week exercise protocol in rats significantly increased heart weight (Burelle et al 2004).

5. Effect of exercise on diabetic hearts

Research associated with glycogen metabolism has been conducted mainly with skeletal muscle; by comparison much less is known about glycogen regulation in cardiac muscle. However, some assumptions can be made in that glycogen is now recognized as an important element in the regulation of glucose metabolism in muscle.

Glycogen exists in two pools that are distinguished on the basis of their solubility in acid. These two types of glycogen are termed proglycogen (acid insoluble) and macroglycogen (acid soluble) and are metabolically distinct in terms of their synthesis and degradation. To date no information exists on the type of glycogen (PG or MG) that is preferentially stored in T1D and T2D mouse hearts, and certainly nothing exists on the type of glycogen in diabetic mouse hearts after a regular exercise program.

5.1 Cardiac glycogen content in diabetic hearts

Studies have shown that as a result of diabetes, cardiac glycogen levels increase significantly compared to nondiabetic hearts (Laughlin et al., 1990; Nakao et al., 1993; Higuchi et al., 1995; Lajoie et al., 2004). The shift toward elevated glycogen storage could occur by initiating the formation of new glycogen granules (PG) and/or by increasing the size of existing glycogen granules (MG) (Alonso et al., 1995; Shearer & Graham, 2002), where glucose residues must be added in tiers around the PG granules, forming larger mature glycogen granules while the number of granules remains constant (Roach, 2002).

Consistent with these data, significant accumulation of glycogen in both T1D and T2D hearts was observed. Total glycogen content was 2-fold greater in unperfused *db/db* SED hearts compared to *db/+* hearts (Figure 13). The increase in glycogen content of T2D SED hearts was due to a statistically significant increase in MG (Figure 12). MG represented 63% of the total amount of glycogen in *db/db* hearts, while *db/+* hearts had only 50% of their total glycogen coming from MG (Figure 12). In other work, a 50:50 ratio of PG to MG was reported in control rat hearts (Alonso et al., 1995). A significant shift in PG:MG in unperfused T2D (*db/db)* hearts demonstrated distinct regulation between MG and PG pools in terms of glycogen synthesis and degradation compared to *db/+* control hearts.

The isolated working heart perfusion with exogenous substrates (11 mM glucose and 0.7 mM fatty acids) produced an unexpected increment in glycogen content of *db/+* SED control hearts. Not only was there a large increase in total glycogen after the 60 min perfusion (Figure 16), but additionally, this accumulation in glycogen was achieved to a large extent by increasing the size of existing glycogen granules (a conversion of PG to MG), resulting in a shift of the 50:50 PG:MG ratio before perfusion to 39:61 after perfusion (Table 3). Thus, in normal healthy control *db/+* SED hearts, glucose is preferentially stored as MG after perfusion suggesting MG is a more dynamic form of glycogen, increasing and decreasing with the ebb and flow of cellular demand. This increase in total glycogen after perfusion in control hearts may reflect recruitment of GLUT-4 transporters to the cell membrane induced by muscle contraction via AMP-activated protein kinase (AMPK), a known energy sensor of the cell when the ratio between AMP/ATP increases. In cardiac muscle, exercise training resulted in a

significant increase in GLUT-4 protein expression and AMPK activity but not PKB in male rats (Coven et al., 2003).

Additionally, total glycogen of T2D hearts was significantly elevated after perfusion, but unlike the *db/+* perfused hearts, *db/db* hearts did not show a shift in the PG:MG ratio (Table 3). Although total glycogen of T2D hearts was significantly elevated after perfusion, the total absolute values were considerably less compared to *db/+* total glycogen levels (Figure 16).

Although T1D data is compromised by having post-perfusion data only, nevertheless there are some interesting findings. Surprisingly, total glycogen values were not significantly different between T1D SED and CD1 SED hearts, contrary to other reported results in T1D rats (Higuchi et al., 1995; Laughlin et al., 1990; Nakao et al., 1993) (Figure 11). Sedentary T1D hearts did have significantly more proglycogen as a percent of total glycogen (59%) compared to control littermates (35%) (Figure 10). Thus, glucose is preferentially stored as PG in cardiac tissue in SED T1D, creating new granules of glycogen. This accelerated rate of PG synthesis was coupled with no increase in MG, indicating PG is a more dynamic fraction of the two pools. Conversely, CD1 control hearts added glucose residues in tiers around existing PG granules, expanding MG as a mechanism of glycogen storage. Interestingly, this same trend was demonstrated by *db/+* control hearts where glucose was preferentially stored as MG after perfusion (Table 3), indicating some commonality between the two strains of healthy control hearts in terms of glycogen storage. It appears the two glycogen pools are metabolically distinct in terms of their synthesis and degradation.

5.2 Significance of elevated glycogen in diabetic hearts

Results from this study showed elevated glycogen levels in diabetic hearts. Typically, this increase in myocardial glycogen has been explained by the preferential utilization of FA by diabetic hearts producing inhibition of glucose utilization (reduction in both glycolysis and glucose oxidation). As a consequence, excess intracellular glucose-6-phosphate is diverted to glycogen synthesis (Laughlin et al., 1990). However, the MG and PG content of diabetic hearts has not been investigated.

In unperfused T2D (*db/db*) hearts, glycogen stores were mainly augmented through conversion of PG to MG. Elevated glycogen concentration in trained T1D hearts was due, in contrast to T2D hearts, to greater increases in new glycogen granule number (PG) rather than expansion of glycogen granule size (MG).

Whether as PG and/or MG, extra glycogen can have negative consequences for the diabetic heart because elevated glycogen may further exacerbate an already deleterious situation produced by a switch in cardiac metabolism with an increase in FA oxidation and a concomitant decrease in glucose utilization (Belke et al., 2000; Aasum et al., 2003; Randle, Priestman, Mistry, & Halsall, 1994b).

Potential mechanisms by which elevated glycogen content promotes metabolic dysfunction in the diabetic heart, although incompletely understood, extend to interference of glycogen with key intracellular signaling pathways, substrate utilization, and glycogen metabolism itself to name a few (Richter & Galbo, 1986). Normally, high glycogen content within the heart will work reciprocally to reduce insulin-stimulated and contraction-induced glucose transport and GLUT-4 translocation to the cell membrane, demonstrating a glycogen storage maximum (Richter & Galbo, 1986; Richter, Derave, &

Wojtaszewski, 2001). Additionally, muscle glycogen content can influence glycogen metabolizing enzymes like glycogen synthase (GS) and their intracellular localization (Nielsen et al., 2001), which in turn exert a regulatory effect on GLUT-4 translocation (Jensen et al., 2006). High glycogen content will reduce GS activity with a concomitant decrease in GLUT-4 translocation (Nielsen et al., 2001). Conversely, when glycogen levels are low glycogen will induce GS to translocate from a glycogen-enriched membrane fraction to a cytoskeleton fraction where glycogen levels are decreased (Nielsen et al., 2001). In summary, low levels of glycogen increase glycogen synthase (GS) activity and GLUT-4 translocation, while conversely high glycogen concentration will reduce GS activity with a concomitant decrease in GLUT-4 translocation (Nielsen et al., 2001). Further, even in the absence of insulin, high glycogen content alone will exert a powerful effect on GS thereby reducing the subsequent rate of glycogen synthesis (Jensen et al., 2006).

Glycogen also has a direct association with several downstream insulin-signaling molecules including protein kinase B (PKB; also called Akt). PKB activation is brought about by dual phosphorylation of Thr^{308} and Ser^{473} residues, by insulin activation of the phosphatidylinositol 3-kinase (PI3K) pathway (Burgering & Coffer, 1995). PKB, in turn, phosphorylates glycogen synthase kinase-3 (GSK-3) leading to inactivation, thereby maintaining GS in a dephosphorylated active state, and hence leading to an acceleration of glycogen synthesis (Cross, Alessi, Cohen, Andjelkovich, & Hemmings, 1995). Elevated glycogen levels will lower PKB expression and activation (Jensen et al., 2006), with concomitant decrease in glycogen synthesis. PKB activity is modulated by glycogen content whereby PKB activity is significantly higher in low glycogen compared with high

glycogen situations. In addition, insulin-stimulated cell surface GLUT-4 content is significantly higher in low glycogen compared with high glycogen, indicating PKB activation is required for metabolic effects of insulin (Derave, Hansen, Lund, Kristiansen, & Richter, 2000).

The relationship between glycogen content and glycogen synthesis exemplifies a homeostatic negative feedback system, suggesting glycogen content has a set point (or transport maximum) about which glycogen levels normally fluctuate, depending upon the need of the cell. However this is not what was observed in the diabetic hearts in this study, which may be explained by a reduction in glucose utilization by the cardiomyocytes due to the preferential utilization of FA by diabetic hearts. Excess intracellular glucose-6-phosphate appears to be diverted to glycogen synthesis, forcing the cell to compromise its original total glycogen concentration set point and adapt to a new phenotype.

Overall, it is this relationship of reciprocity between glycogen content and key signaling pathways, substrate uptake, and glycogen metabolism that drives the rate of glycogen synthesis up or down. This was not the case for a diabetic heart. Elevated glycogen concentrations have been suggested as a causal factor in metabolic dysfunction in the diabetic heart (Jensen et al., 2006). Given this and the results from this study, elevated diabetes-induced glycogen levels may contribute to or perpetuate impaired glucose utilization by interfering with insulin signaling, enzyme activity and regulation by subcellular translocation. The actual mechanism by which high glycogen content disrupts the negative feedback system to amplify glycogen synthesis is currently not known. But whatever the mechanism, the response to diabetes enhances or exaggerates

the original stimulus so that the activity (increased glycogen content) is accelerated creating a positive feedback loop, thus causing the variable (high glycogen content) to deviate further and further from its original value or range.

In addition to observed elevated glycogen levels in diabetic hearts, it was obvious perfused T2D hearts exhibited a reduction in glycogen synthesis compared to perfused *db/+* control hearts (Figure 16). These observations suggest impaired glucose utilization by *db/db* hearts; either an upper glycogen storage limit is reached or alternatively, the rate of glycogen synthesis may proceed at a much slower rate in *db/db* hearts. Additionally, a significant shift in PG:MG in unperfused T2D hearts demonstrates distinct regulation between MG and PG pools in terms of glycogen synthesis and degradation compared to *db/+* control hearts. Further, the differences found between T1D and T2D hearts in their type of glycogen and PG:MG distribution suggests not only is there is an alternative regulatory mechanism behind glycogen use and storage between type 1 and type 2 diabetic mouse hearts, but creating new granules may be a compensatory strategy in T1D cardiac tissue where insulin resistance comes from mice with insulin deficiency.

Some have suggested there is a silver lining in diabetes-induced high myocardial glycogen levels. Patients with diabetes are more prone to congestive heart failure. As a diabetic cardiomyopathy progresses into heart failure, the heart becomes ischemic (Kannel & McGee, 1979). An ischemic heart can metabolize glycogen without producing elevated hydrogen ion content as is the case when exogenous glucose is used for metabolism (Ravingerova et al., 2000). Thus elevated glycogen may exert a cardioprotective mechanism during heart failure (Henning, Wambolt, Schonekess, Lopaschuk, & Allard, 1996; Finegan, Lopaschuk, Gandhi, & Clanachan, 1995).

5.3 Effect of exercise on the glycogen content of diabetic hearts

Perfused T1D (Figure 11) and unperfused T2D hearts (Figure 13), but not perfused T2D hearts (Figure 16), were clearly responsive to a distinct effect of exercise as determined by significantly elevated total glycogen compared to their respective SED diabetic hearts. However, at no time did the PG:MG ratio change in response to exercise in any of the diabetic hearts (Figures 10 and 12).

Results indicated that T1D SED hearts exhibited increased glycogen content by exhibiting a propensity toward PG storage, while MG storage was somewhat blunted compared to CD1 hearts. T1D EX hearts exploited this tendency of preferential PG storage further; an even higher amount of PG was observed. However, T1D EX hearts not only created new glycogen granules in the form of PG, but additionally were able to add glucose residues to existing granules thus making more MG. In T1D EX hearts, increased total glycogen was due to significant increases in both PG and MG (Figure 10) compared to T1D SED hearts. T1D EX MG levels were not significantly different than CD1 control hearts (Figure 10).

Unperfused T2D EX hearts were also sensitive to the effects of exercise, and demonstrated a similar trend as T1D EX hearts. Both PG and MG were significantly elevated compared to T2D SED hearts (Figure 12), and therefore total glycogen levels were significantly higher (Figure 13). PG:MG ratios did not change with exercise; PG as a per cent of total glycogen remained at 36 – 37% (Figure 12).

The effect of exercise was no longer evident in T2D EX hearts after perfusion; total glycogen content, was no longer significantly different from T2D SED hearts (Figure 16). This observation suggests that a metabolic impairment attributable to

diabetes was revealed after perfusion. Sedentary T2D hearts increased their total glycogen content by 14 glucosyl units (μmol/g) after perfusion (a 50% increase), while trained T2D hearts increased glycogen content by 8 glucosyl units (μmol/g) compared to pre-perfusion hearts (a 27% increase). To put this in perspective, *db/+* control hearts increased their total glycogen levels by approximately 36-40 glucosyl units (μmol/g), an 83%-84% increase. Thus, exercise did not attenuate an altered glycogen metabolism in T2D hearts.

Elevated glycogen brought about by training in diabetic hearts may be explained, in part, by an exercise-induced increase in GLUT-4 translocation into the cardiomyocyte membrane in diabetic hearts (Hall et al., 1995; Osborn et al., 1997). The insulin activated GLUT-4 signaling pathway has been shown to be defective in diabetic humans and animals (Hall et al., 1995; Avogaro et al., 1990; Garvey et al., 1993; Semeniuk et al., 2002; Huisamen & Lochner, 2005; Penpargkul et al., 1980). Training-induced GLUT-4 translocation in diabetic hearts and the resultant increased glucose entry will increase intracellular glucose-6-phosphate which is diverted to glycogen synthesis (Laughlin et al., 1990) because glycolysis and glucose oxidation are impaired in diabetic hearts. Given that elevated glycogen exacerbates metabolic dysfunction (Jensen et al., 2006), the exercise-induced increment in cardiac glycogen in T1D and T2D hearts should have further negative consequences for the diabetic heart.

5.4 Effect of diabetes on palmitate oxidation rates

A key objective of this study was to study exercise-induced changes in cardiac function and metabolism in T1D and T2D mice assessed with *ex vivo* perfused hearts.

Therefore, in addition to glycogen content, the oxidation of radiolabeled palmitate (complexed to albumin) was measured.

In accordance with previous studies with other type1 diabetic (*stz*-induced) animals (Stanley, Lopaschuk, & McCormack, 1997; Neitzel et al., 2003; Christe & Rodgers, 1995), basal rates of FA oxidation were elevated in T1D SED hearts compared to control CD1 SED hearts (Figure 19). Surprisingly, FA oxidation rates were not significantly elevated in T2D SED hearts (Figure 20) compared to control *db/+* SED hearts. These results for *db/db* hearts are not consistent with previous research which has demonstrated elevated rates of FA oxidation in perfused *db/*db hearts (Aasum et al., 2003; Hafstad et al., 2006; Carley et al., 2004).

The protocol for measuring FA oxidation in this thesis research was not different from (Neitzel et al., 2003) and (Carley et al., 2004) in the same Severson lab. To ensure each heart was functioning at physiologically relevant workloads during perfusion, preload and afterload were re-measured continually throughout the study. In addition, when hearts did not perform with consistent expected flow rates, the rig apparatus was cleaned by soaking in an acid and base, to remove any contaminating debris. Two *db/db* mice were sent to pathology for microbial testing as *Klebsiella pneumoniae*, a cardiopulmonary disease-causing bacterium, has been associated with Jackson Laboratory mice but the pathology reports came back negative. One other variable remained which was beyond our control, and that was the on-going construction next to the mouse vivarium. Other investigators had reported odd behaviours and unusual findings in their mouse-related experiments at this same period. Subtle noise, vibrations

and noxious fumes from the construction may have played a role in the well-being of our mice and therefore contributed to unexpected FA oxidation results with *db/db* hearts.

5.5 Effect of exercise on FA oxidation by diabetic hearts

The elevated FA oxidation rates in T1D SED hearts (relative to CD1 SED hearts) were not significantly different from T1D EX hearts (Figure 19). Therefore, the training protocol did not alter the diabetic phenotype of elevated FA oxidation by T1D mouse hearts, contrary to the hypothesis. Because fatty acid oxidation rates were not elevated in SED T2D mice, it was not possible to see any effect of training on fatty acid oxidation rates in T2D EX hearts.

Several factors could account for the observation that exercise did not attenuate FA oxidation in diabetic hearts used in this study: 1) incorrect hypothesis that exercise will decrease FA oxidation when exercise training is known to increase FA uptake and oxidation in normal hearts (Burrelle et al 2004); 2) insufficient exercise volume; 3) animal model discrepancy, and 4) mice were too diabetic to begin with, as evidenced by their extremely high blood glucose levels.

One of the classic effects of aerobic endurance training is an increase in the oxidative capacity of muscle resulting in a shift toward greater FA oxidation (Achten & Jeukendrup, 2004; Coyle, 1995). A study by Burelle et al. (2004) in which trained *ex vivo* rat hearts exhibited an increased FA oxidation rate affirms this in the rat heart. Interestingly, in diabetes (Rodrigues & McNeill, 1992; Stanley, Lopaschuk, Hall, & McCormack, 1997) and endurance training, the metabolic consequence in both of these situations is an increase in myocardial FA oxidation when each is examined independently. When exercise training was superimposed on diabetic mouse hearts, there

was no change in FA oxidation rates. To explain these results, the cellular FA utilization pathway must be examined in terms of diabetes and compared to expected changes during endurance training.

In diabetes, initial elevated plasma FA levels are a result of insulin deficiency or resistance, while increased plasma FA levels during training come mainly from exercise-induced lipolysis in adipose tissue (Coyle, 1995). Not only does insulin deficiency/resistance lead to increased FA levels but there is a corresponding increase in uptake of FA into the heart (Schaffer, 2003; Unger, 2002). This is accomplished by increased localization or redistribution of FAT/CD36 to the plasma membrane (Coort et al., 2004; Luiken et al., 2001). The consequence of increased FA uptake typically results in enhanced FA oxidation in diabetic hearts with concomitant depressed glucose oxidation. Elevated FA oxidation rates in diabetic hearts have been demonstrated (Wang, Lloyd, Zeng, Bonen, & Chatham, 2005; Belke et al., 1999). Exercise and muscle contraction can increase protein expression of FA transporters and mitochondrial CPT1 in response to a training stimulus (Guzman & Castro, 1988; Bonen, Dyck, Ibrahimi, & Abumrad, 1999; Turcotte, Swenberger, Tucker, & Yee, 1999) with a consequent increase in FA oxidation (Burelle et al., 2004). However, when exercise training was superimposed on T1D and T2D diabetic mouse hearts, there was no change in FA oxidation measured with an *ex vivo* isolated perfused heart model.

If elevated FA oxidation rates are not attenuated with exercise, another fate for intracellular fatty acyl-CoA is conversion to TG. If this is the case, then this suggests training may exacerbate an existing problem by routing fatty acyl CoA to intracellular storage, thus contributing to lipotoxicity. It has been shown in diabetic hearts that FA

over-load can be re-directed to intracellular storage with concomitant contractile dysfunction (Young et al., 2002a). Elevated levels of FA are known to be lipotoxic with deleterious consequences for heart function (Unger, 2002). Recently, Searls et al. (2004) have provided evidence that exercise does not reduce lipid accumulation in cardiomyocytes; however, again, their exercise protocol was different than this study. T1D rats were exercised for two weeks before they were injected with streptozotocin intraperitoneally. Pre-training may have had some effect on intracellular TG mobilization in the heart. However, assuming exercise does not decrease intracellular lipid storage and diabetes causes increased lipid accumulation, there is reason to speculate that moderate intensity exercise may not attenuate lipotoxicity in the diabetic heart.

Metabolic fitness has been proposed as a method of training that replaces traditional cardiorespiratory fitness (Despres & Lamarche, 1994) for greater health-benefits. Traditional cardiorespiratory fitness emphasizes high-intensity, vigorous exercise aimed at increasing VO_2max , an attained physiological state, while metabolic fitness is geared to produce a substantial increase in daily energy expenditure, a behaviour that will lead to improvements in carbohydrate and lipid metabolism, metabolic variables associated with CHD and diabetes. Improving or maintaining glucose and lipid homeostasis occurs through participation in an adequate dose of physical activity on a regular basis (Albright et al., 2000). Training at this intensity, which represents between 40 – 65% of VO_2max, is considered an intensity that reduces the reliance on carbohydrate as an energy source, thereby increasing fat oxidation during submaximal exercise (Achten & Jeukendrup, 2004; Romijn et al., 1993). The training protocol used in this study was low-moderate intensity, where mice trained for one hour

per day, 5 days per week for 6 weeks. This same exercise training program attenuated endothelial dysfunction in T2D (*db/db*) mice (Howarth, personal communication) but clearly did not lower FA oxidation rates in T2D (*db/db*) or T1D mouse hearts. Several important questions need to be asked. At what exercise intensity can the highest rates of fat oxidation be found? And, is training at the highest rate of fat oxidation appropriate for a diabetic pathology? For example, will low-moderate training intensities target impaired lipid metabolism since at these training intensities maximal fat oxidation during exercise occurs? Or, will maximal fat oxidation with training only exacerbate lipid accumulation in the insulin resistant cardiomyocyte as outlined above? Perhaps the real target with exercise training in treating diabetes is the utilization of carbohydrates at training intensities held above 65% VO_2max, where glycogen and exogenous glucose are the main substrates for fuel.

The other consideration with training intensity and volume is that the total duration of the training (6 weeks) may not have been long enough to impact FA oxidation rates; but again, what is an adequate dose of physical activity that will improve and maintain glucose and lipid homeostasis? There are no definitive answers in the literature. Finally, researchers have found physical training between 40 – 65% improves lipid and lipoprotein profiles (Halbert, Silagy, Finucane, Withers, & Hamdorf, 1999; Bassuk & Manson, 2005); however these are metabolic risk factors closely associated with CHD and atherosclerosis, whereas diabetic cardiomyopathy has other risk factors.

5.6 Effect of exercise on contractile function in control and diabetic hearts

Control *db/+* mouse hearts showed no changes with exercise for any of the measured parameters of contractile function (Table 5). Studies conducted on isolated

working hearts from normal trained rats using a comparable training protocol to the one used in this study have not demonstrated significant changes in cardiac function with exercise (Bowles et al., 1992; Broderick et al., 2004; Paulson et al., 1987; De Angelis et al., 2000). Trained CD1 control hearts unexpectedly exhibited a marked increase in cardiac power (CO * PSP mmHg·ml/min). Burelle et al. (2004) did demonstrate significant differences between control sedentary and exercised rat hearts for hydraulic power which was 25% higher in trained control hearts because of an increase in cardiac output; however, this difference disappeared after normalization for heart weight. The possible explanation for the unexpected increase in hydraulic power in this study may be attributed to a higher (although not significant) aortic flow rate. It's difficult to imagine there is real significance in this contractile parameter when training intensity was so minimal. Possible measurement error, small sample size or a difference between mice and rats when it comes to contractile function and the effects of exercise may be contributing factors.

Assessment of diabetic cardiomyopathy using rodents has come mainly from *stz*-injected or alloxan-treated T1D rat studies. The cardiovascular consequences of *stz*-induced diabetes in rats have been described in detail (Tomlinson et al., 1992; Rodrigues, Cam, & McNeill, 1995; Taegtmeyer, McNulty, & Young, 2002; Young et al., 2002a). The T1D rat presents significant hyperglycemia with significant reductions in heart rate and diastolic performance. Systolic dysfunction (cardiac output) is detected but occurs later in the course of the disease (Mihm et al., 2001; Christe & Rodgers, 1995). Isolated working hearts from obese Zucker *fa/fa* rats, a model of obesity and insulin resistance,

show depressed contractile function where cardiac power was reduced compared with the lean Zucker rat (Young et al., 2002a) .

The T1D and T2D SED hearts did not exhibit any degree of cardiovascular dysfunction, which was an unexpected result. There is evidence from both *in vivo* and *ex vivo* analyses that demonstrates cardiac dysfunction in hearts from both *stz*-treated T1D and *db/db* mice (Neitzel et al., 2003; Nielsen, Bartels, & Bollano, 2002; Belke et al., 2000; Aasum et al., 2002; Semeniuk et al., 2002; Carley et al., 2004). There are limitations in the indices of contractile function that were utilized in this study, namely timed collection of aortic and coronary flows and PSP measurements in the aortic afterload line. More sophisticated methods of monitoring contractile function (pressure-volume loop analysis, for example) including recovery of contractile function after ischemia-reperfusion may have revealed contractile dysfunction in the perfused hearts from T1D and T2D mouse models used in this study.

Trained T1D hearts presented an unusual finding in that RPP was significantly reduced when compared to SED T1D hearts (Figure 22). The RPP has been used as an effective means of measuring myocardial mechanical performance (Burelle et al., 2004). RPP decreased due to a corresponding significant decrease in heart rate (Table 5, Figure 21). A decrease in RPP in EX T1D hearts suggests exercise has had an adverse effect on the function of the heart. This result seems contrary to what is expected as exercise normally improves cardiac function. Unfortunately, there are no other isolated working heart studies that have utilized an exercise intervention. Paced T1D EX and T1D SED hearts could be used to remove any heart rate changes.

6. Summary of significant findings

1. Exercise decreased the profound hyperglycemia that characterizes T1D and T2D mice. These changes are most likely a consequence of exercise-induced intracellular responses to increased GLUT-4 translocation to the plasma membrane of skeletal muscle.
2. Exercise further enhanced diabetes-induced myocardial glycogen content in T1D perfused hearts and T2D unperfused hearts by accelerating glycogen synthesis.
3. There was no effect of exercise on glycogen content in T2D perfused hearts; however, metabolic impairment attributable to diabetes remained which was revealed after perfusion.
4. Exercise did not alter rates of FA oxidation in diabetic hearts. Excess fatty acyl CoA could be stored in the cardiomyocyte, further exacerbating lipotoxicity.
5. Exercise did not improve contractile function in diabetic hearts.

These findings that exercise may exacerbate diabetes must be tempered by pointing out only a single exercise condition using animal models with the animal model limitations noted above was examined.

7. Future directions

The real advantage of exercise as a therapeutic benefit for diabetic individuals may reveal itself during ischemic-reperfusion incidents. Mouse hearts are much more sensitive to ischemia-reperfusion damage compared to other species. It is this sensitivity to ischemic injury that can provide valuable information about recovery of contractile functions after no-flow or low-flow ischemia. Endurance training may confer protection

against ischemia and reperfusion-induced injury. This has been demonstrated in normal trained rats (Burelle et al., 2004); RPP improved to a greater degree compared to sedentary rats after ischemia-reperfusion induced injury. Therefore future experiments should look at the effects of exercise after ischemia-reperfusion injury in *ex vivo* perfused working diabetic mouse hearts.

Cardiac energy shifts occur as a response to normal physiologic, pathologic and exercise conditions. Future studies should determine if FA uptake is elevated in the *stz*-induced T1D and T2D (*db/db*) trained hearts by quantifying PPARα expression, FAT/CD36, FABPpm, ACS, MCD and CPT1 protein expression. As well, does exercise cause a redistribution of FAT/CD36 to the plasma membrane?

An exercise program geared toward attenuating diabetes-induced cardiomyopathy must consist of an appropriate exercise training paradigm, including training duration and intensity. These primary training factors control the response, i.e., 'metabolic fitness'. By selecting several exercise intensities (for example, 25%, 50% and > 65% of VO_2max) and various durations of training, evaluation of FA and glucose metabolism (including evaluation of type and molecular nature of glycogen accumulation) utilized at these training regimens can be determined using *ex vivo* perfused heart model to quantify the effects of exercise on cardiac metabolism by diabetic mouse hearts. Both perfused and unperfused glycogen accumulations should be ascertained to make up for the short-fall within this study.

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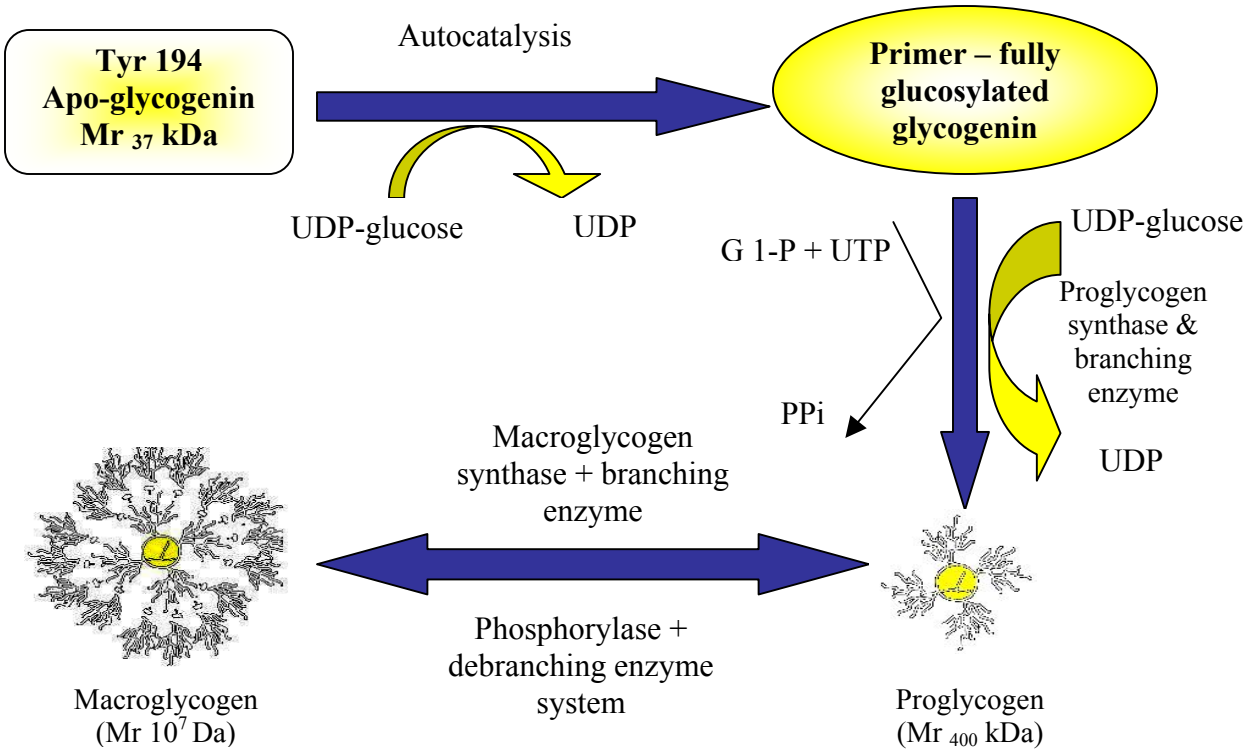

Figure 1. The reversible conversion of glycogenin into macroglycogen, via proglycogen. Glycogenin autocatalytically adds glucose from UDP-glucose to its Tyr- 194, then subsequent apposition of 1,4-α-linked glucose residues to the first residue, such that an average of 8 glucose residues is added. This forms a primer for the synthesis of proglycogen by proglycogen synthase. Branching enzyme is also required. A pool of proglycogen accumulates that is converted to macroglycogen, using macroglycogen synthase. Branching enzyme is required again. Macroglycogen breakdown, catalyzed by phosphorylase and the debranching enzyme system, proceeds to proglycogen, or will continue to glycogenin, but degradation ceases when about four residues remain. Figure is adapted from (Alonso et al., 1995).

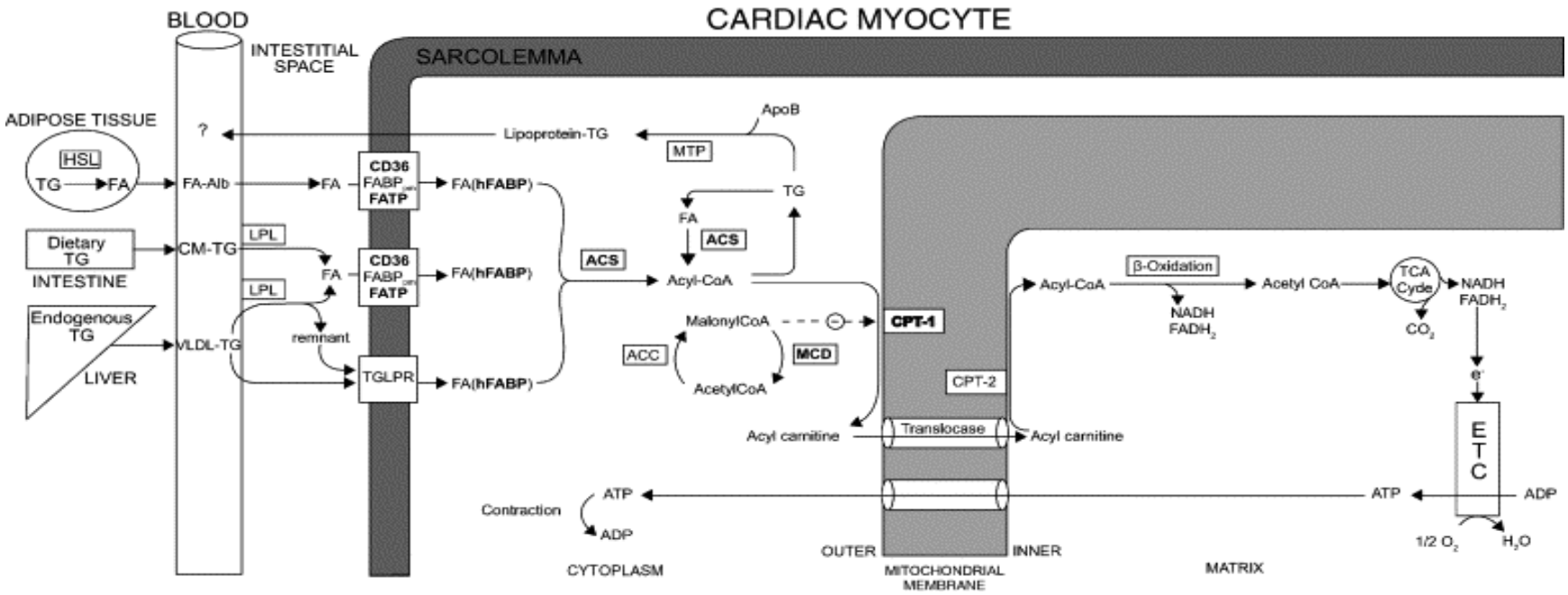

Figure 2. Cardiac FA metabolism. There are two exogenous FA sources in the circulation that are available for cardiac utilization: (a) adipose tissue lipolysis (catalyzed by hormone-sensitive lipase, HSL) produces circulating FA complexed to plasma albumin (Alb); and (b) uptake of triacylglycerols (TG) in circulating lipoproteins, either by the hydrolytic action of an enzyme, lipoprotein lipase (LPL) located on the endothelium of the coronary vasculature, producing LPL-derived FA, or by a lipoprotein receptor-mediated pathway (TGLPR). Chylomicrons (CM) transport dietary TG, whereas very-low-density lipoproteins (VLDL) transport endogenous TG formed in the liver. Intracellular FA metabolism involves formation of fatty acyl CoA and then either esterification to TG or entry into mitochondria (CPT-1-dependent) for β-oxidation and energy production by the electron transport chain (ETC) and oxidative phosphorylation. Activity of CPT-1 is regulated by malonyl CoA content, determined by the net effects of synthesis (ACC) and degradation (MCD). Some cardiac PPARα targets (enhanced expression) are shown in bold. Figure is from (Carley & Severson, 2005).

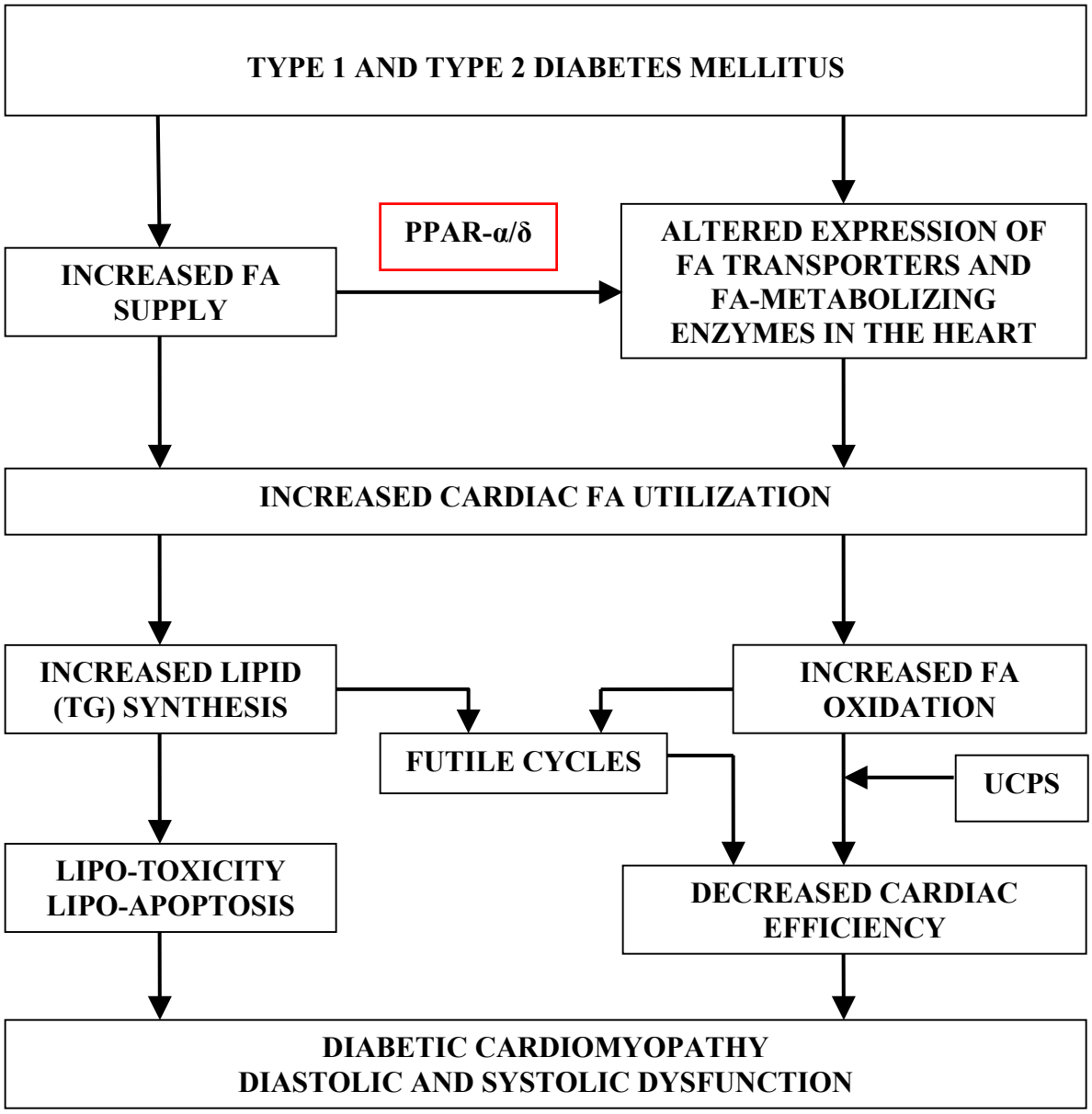

Figure 3. Abbreviations: FA, fatty acid(s); PPAR – α/δ, peroxisome proliferator-activated receptors- α/δ; TG, triacylglycerols; UCPs, mitochondrial uncoupling proteins.

Increased FA utilization in diabetic hearts can produce contractile dysfunction by two mechanisms:

(a) increased lipid synthesis and lipotoxicity (lipoapoptosis)

(b) decreased cardiac efficiency because of elevated rates of FA oxidation and futile cycles.

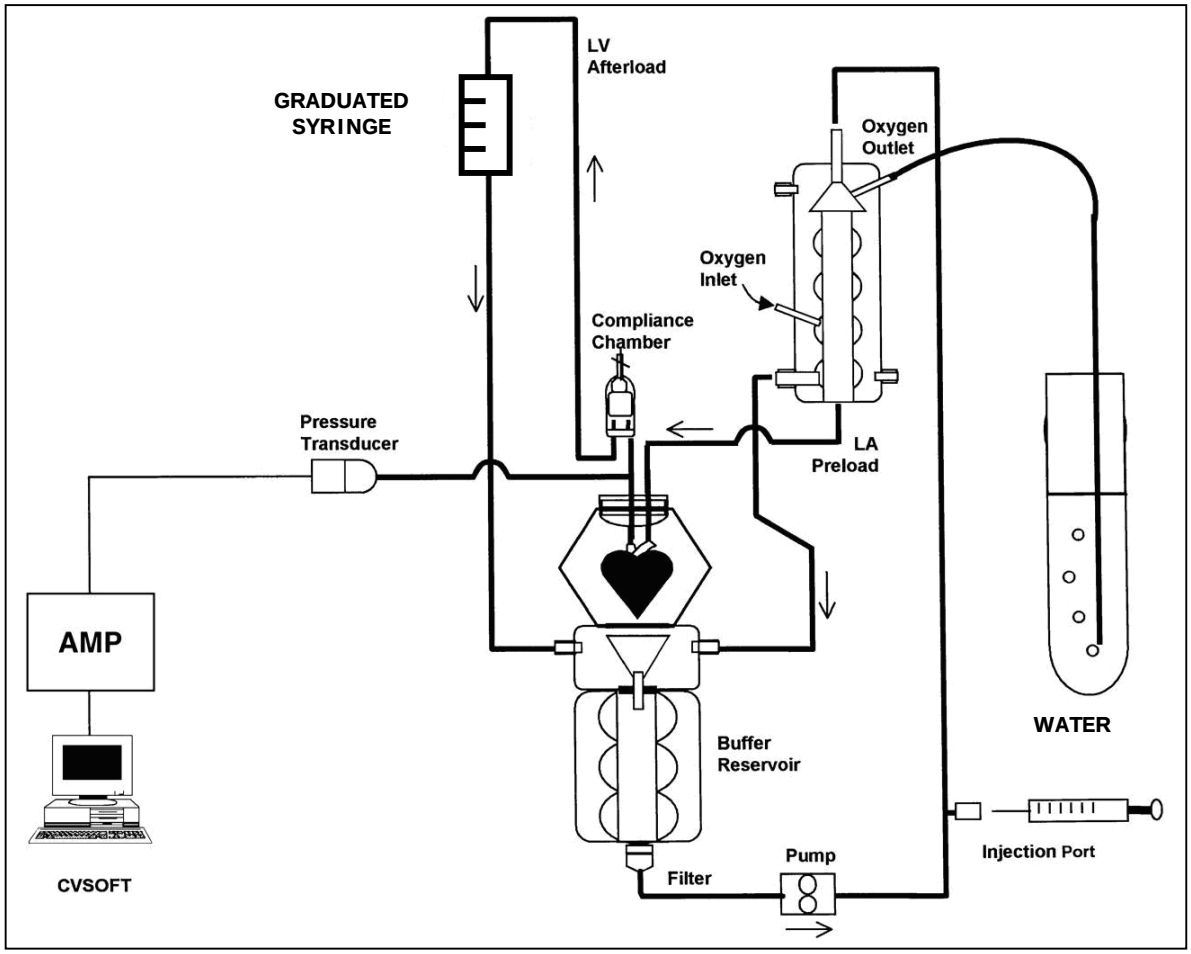

Figure 4.The working heart apparatus. Heart contractility (peak systolic pressure) and heart rates were measured with a pressure transducer in the aortic after-load line (Millar Micro-tip, Millar Instruments). Aortic pressure traces and heart rate were recorded with CV Works software. Aortic flow rates were measured by a timed collection from the flow in the afterload line. The dripping pulmonary artery perfusate constitutes coronary flow. The sum of coronary and aortic flows represents cardiac output. Palmitate substrate was added to the recirculating buffer, and buffer samples were removed through the injection port at 20-minute intervals. Figure is adapted from Belke et al. (1999).

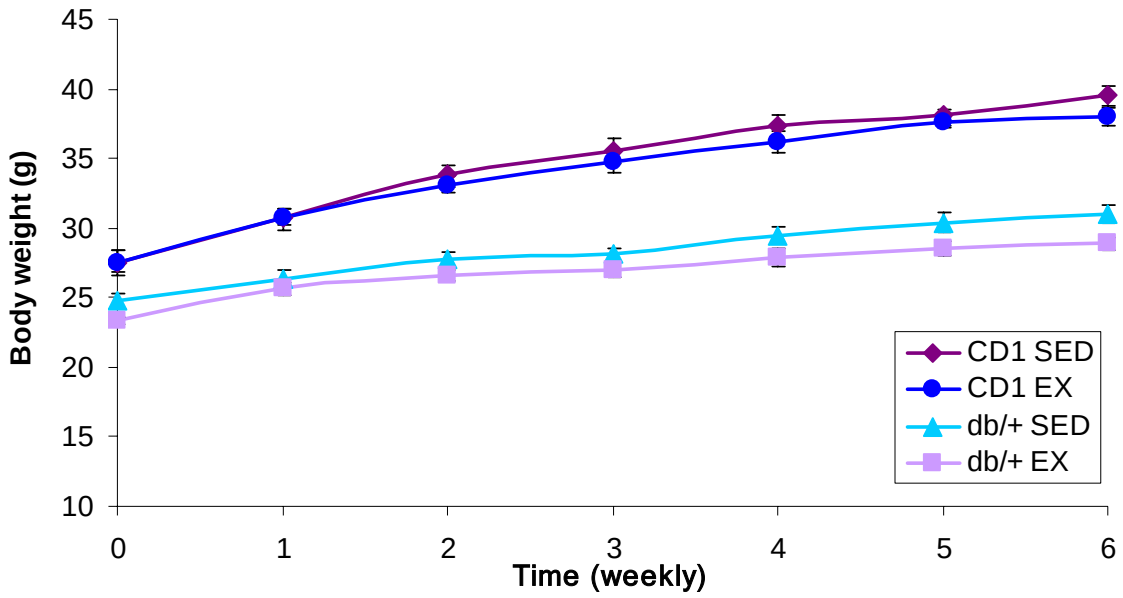

Figure 5. Weekly body weight changes for CD1 and *db/+* control mice: sedentary (SED) groups and during 6 weeks of exercise training (EX groups). N = 8 per group. There were no significant differences between EX and SED weight changes over time.

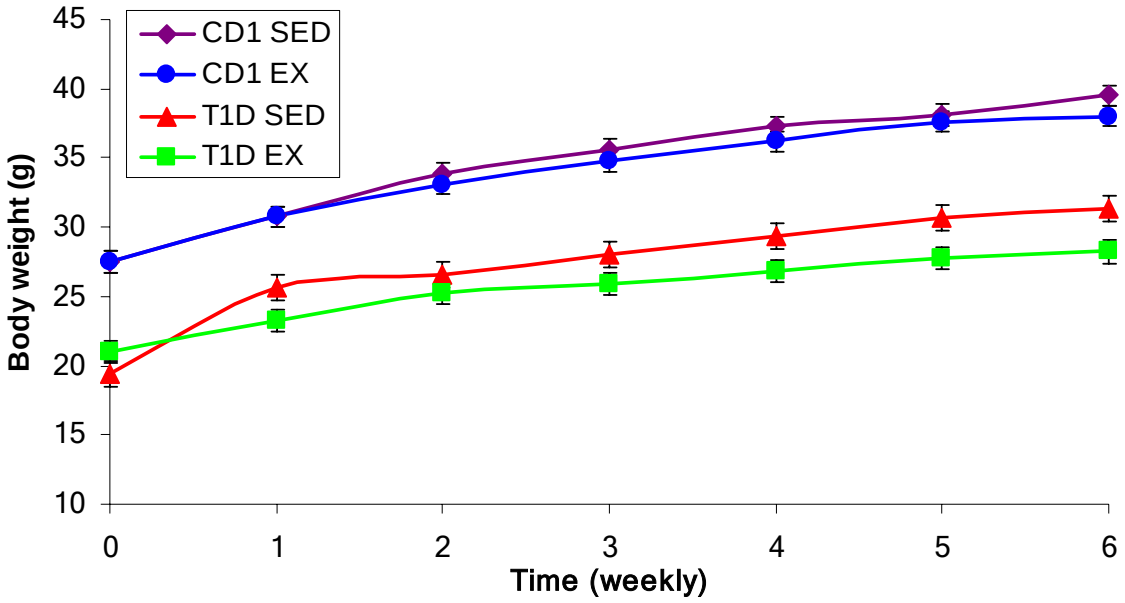

Figure 6. Average weekly body weight changes for CD1 controls and T1D mice: sedentary (SED) groups and during 6 weeks of training (EX groups). No significant weight differences were evident between exercise and sedentary conditions for either CD1 or T1D mice. N = 8 / group.

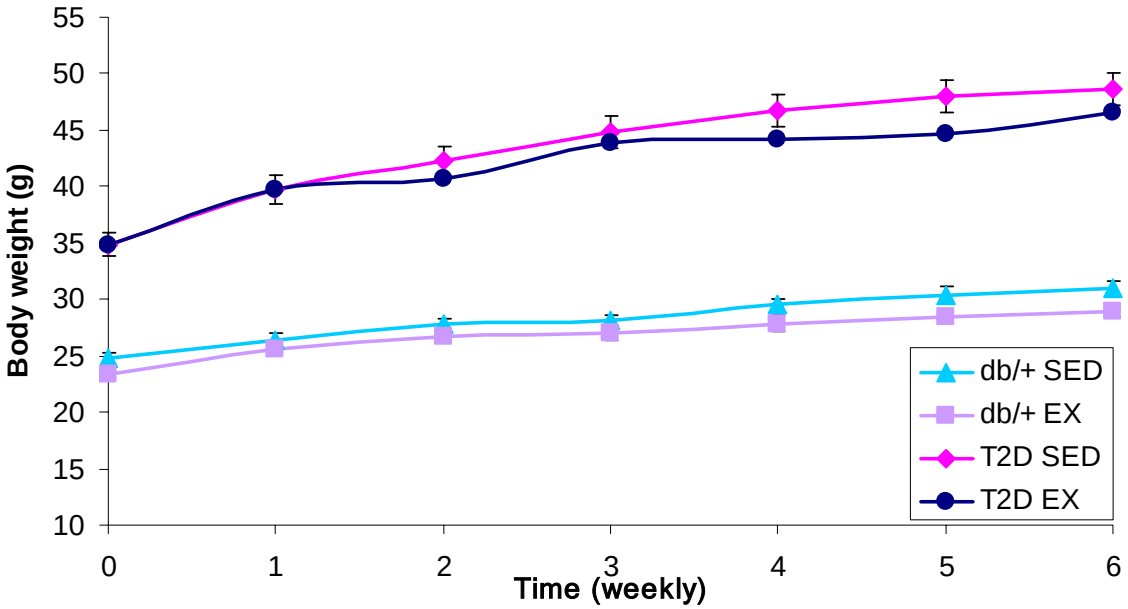

Figure 7. Average weekly body weight changes for *db/+* and T2D (*db/db*) mice: sedentary (SED) groups and during a 6 week exercise training program (EX groups). No significant weight differences were observed between exercise and sedentary treatments for either *db/+* or T2D mice. N = 8.

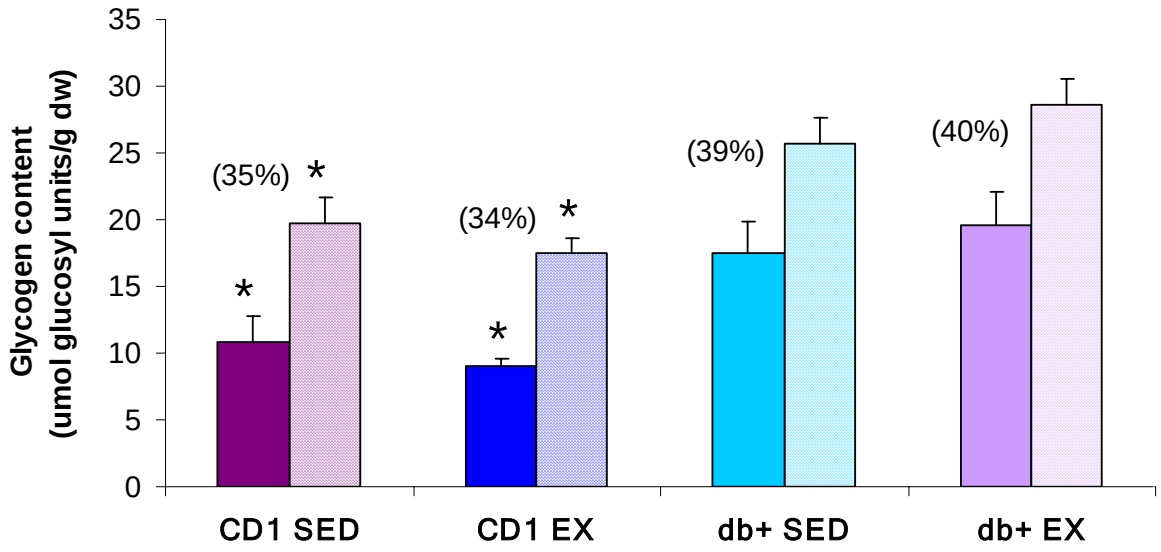

Figure 8. Proglycogen (solid bars) and macroglycogen (hatched bars) content of hearts from non-diabetic CD1 and *db*/+ mice comparing trained (EX) and untrained (SED) conditions. Proglycogen content as a % of total is indicated above each treatment.

* , $p<0.05$ vs. db/+ controls

CD1 SED (N = 7); CD1 EX (N = 7); *db*/+ SED (N = 20); *db*/+ EX (N = 11)

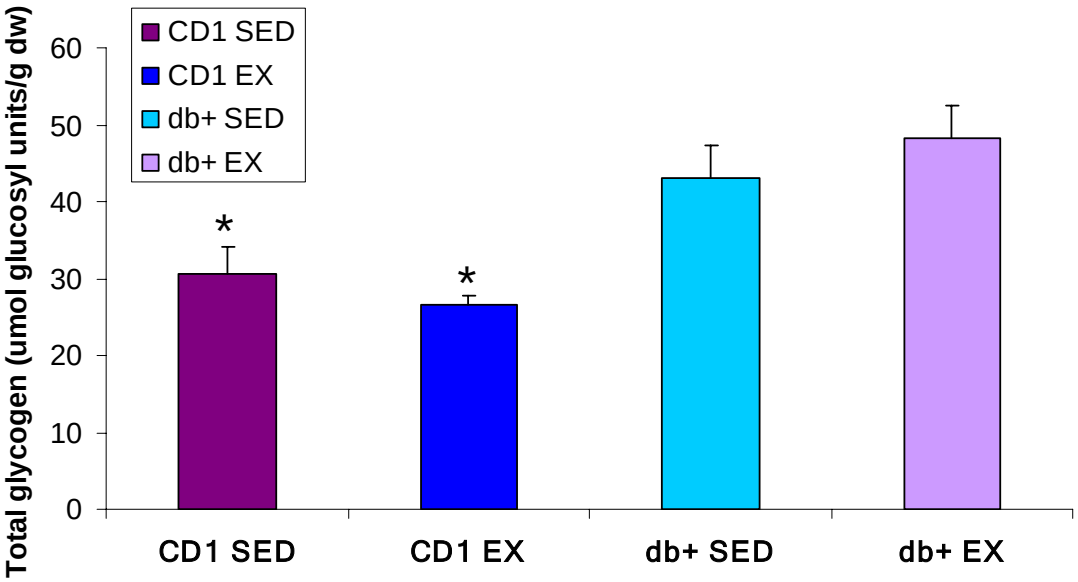

Figure 9. Total glycogen content of hearts from control CD1 and *db*/+ mice comparing trained (EX) and untrained (SED) conditions. *, $p<0.05$ vs. *db*/+.
CD1 SED (N = 7); CD1 EX (N = 7); *db*/+ SED (N = 20); *db*/+ EX (N = 11)

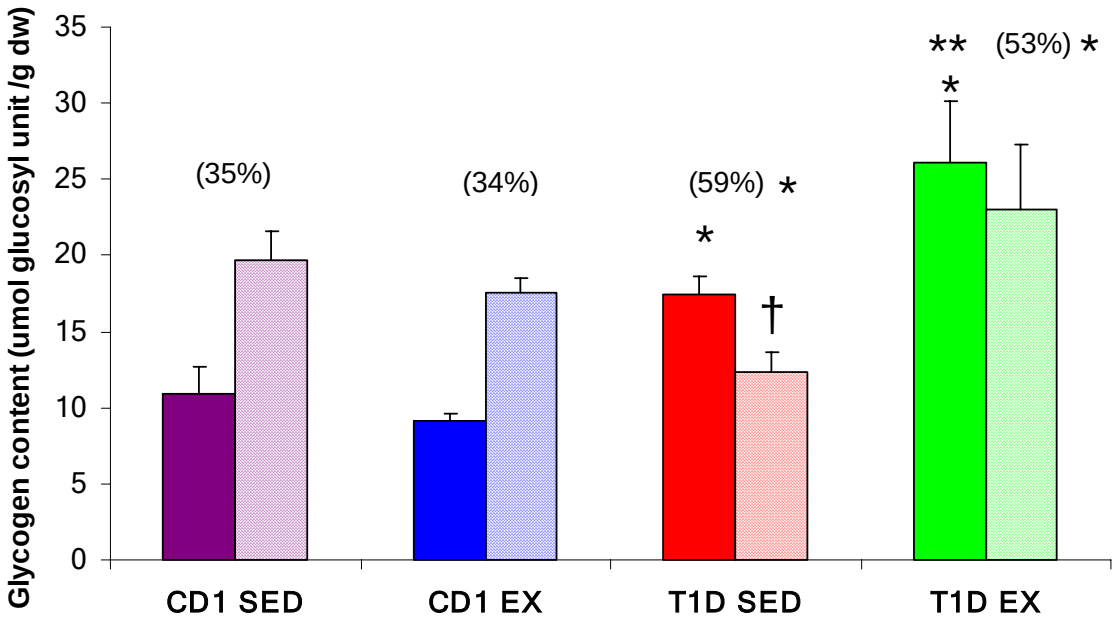

Figure 10. Proglycogen (solid bars) and macroglycogen (hatched bars) content of perfused hearts from control CD1 and T1D (stz-injected) mice, comparing trained (EX) and untrained (SED) conditions. Proglycogen content as a % of total is indicated above each treatment.

* , $p<0.05$ vs CD1 hearts; ** , $p<0.05$ T1D EX vs.T1D SED; †, $p<0.05$ T1D SED vs CD1 SED and T1D EX.

CD1 SED (N = 7); CD1 EX (N = 7); T1D SED (N = 6); T1D EX (N = 6)

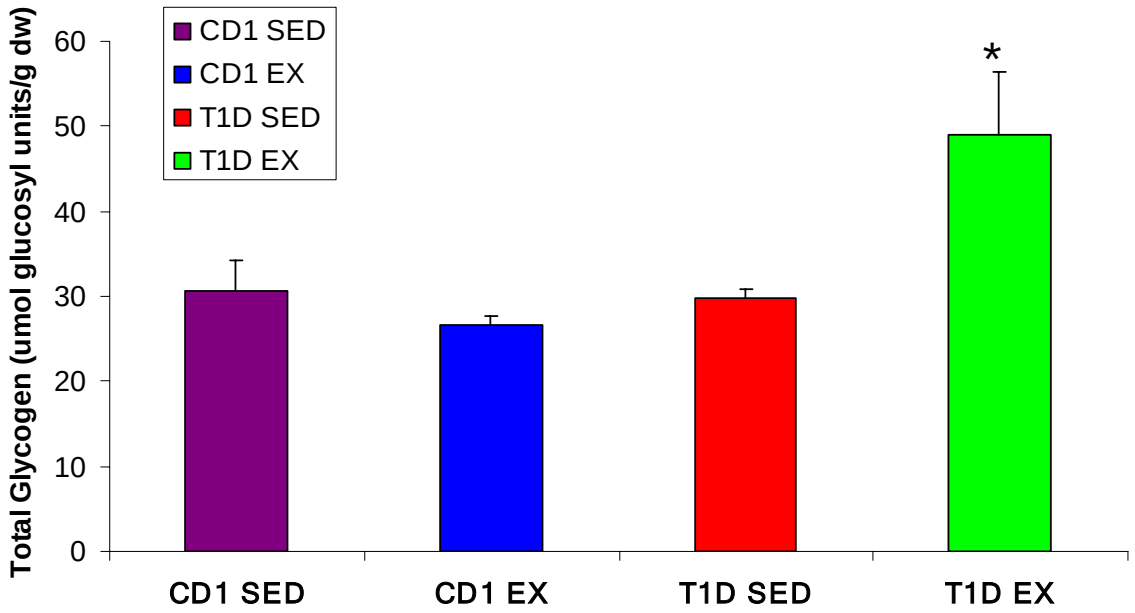

Figure 11. Total glycogen content of perfused hearts from non-diabetic CD1 and T1D (stz-injected) mice, comparing trained (EX) and untrained (SED) conditions.

*, $p<0.05$ vs all other treatments.

CD1 SED (N = 7); CD1 EX (N = 7) ; T1D SED (N = 6) ; T1D EX (N = 6)

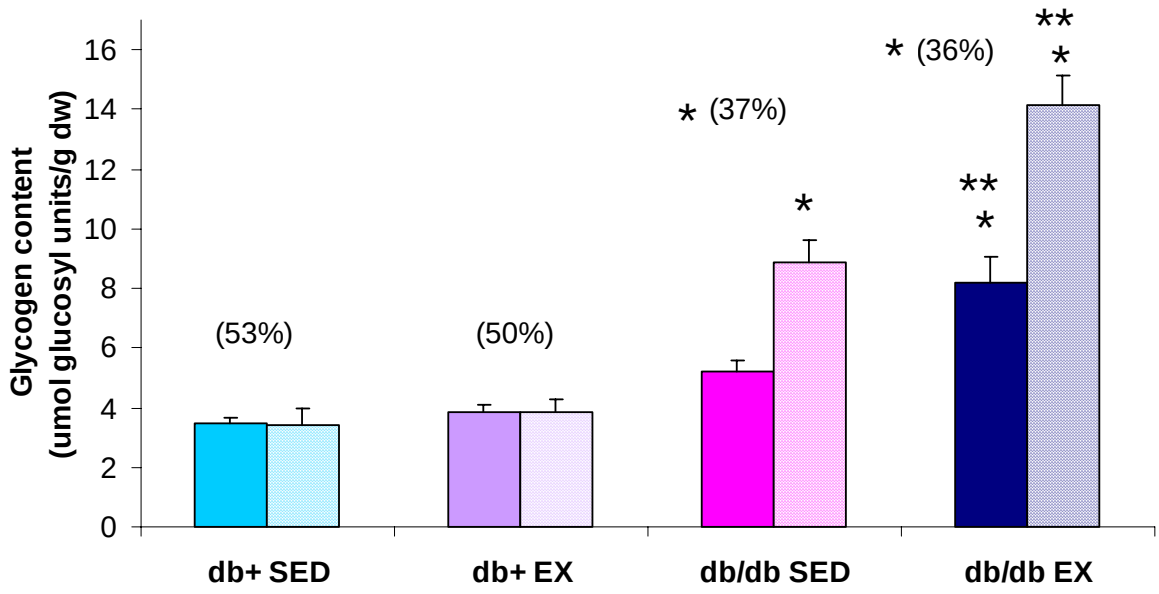

Figure 12. Proglycogen (solid bars) and macroglycogen content (hatched bars) of unperfused hearts from control (*db/+)* and diabetic (*db/db)* mice, comparing trained (EX) and untrained (SED) conditions. Proglycogen content as a % of total is indicated above each treatment.

* , $p<0.05$ vs *db/+* controls; **, $p<0.05$ *db/db* EX vs *db/db* SED.

N = 5 / group.

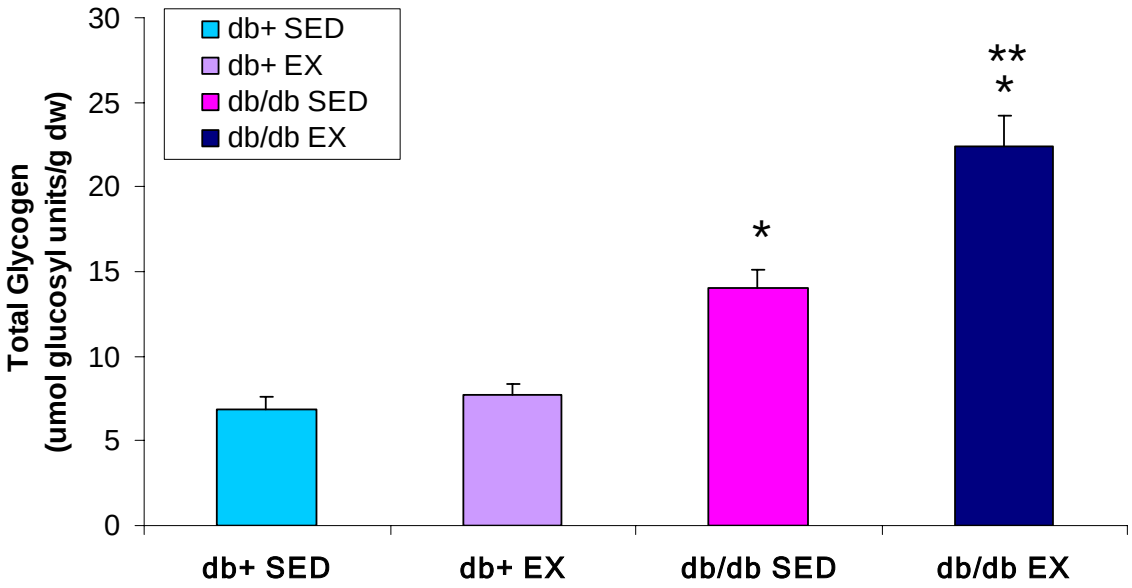

Figure 13. Total glycogen content of unperfused hearts from control (*db/+*) and C57BL/KsJ-diabetic (*db/db*) mice, comparing trained (EX) and untrained (SED) conditions.

*, $p<0.05$ vs. *db/+* controls; ** , $p< 0.05$ vs *db/db* SED.

N = 5 / group

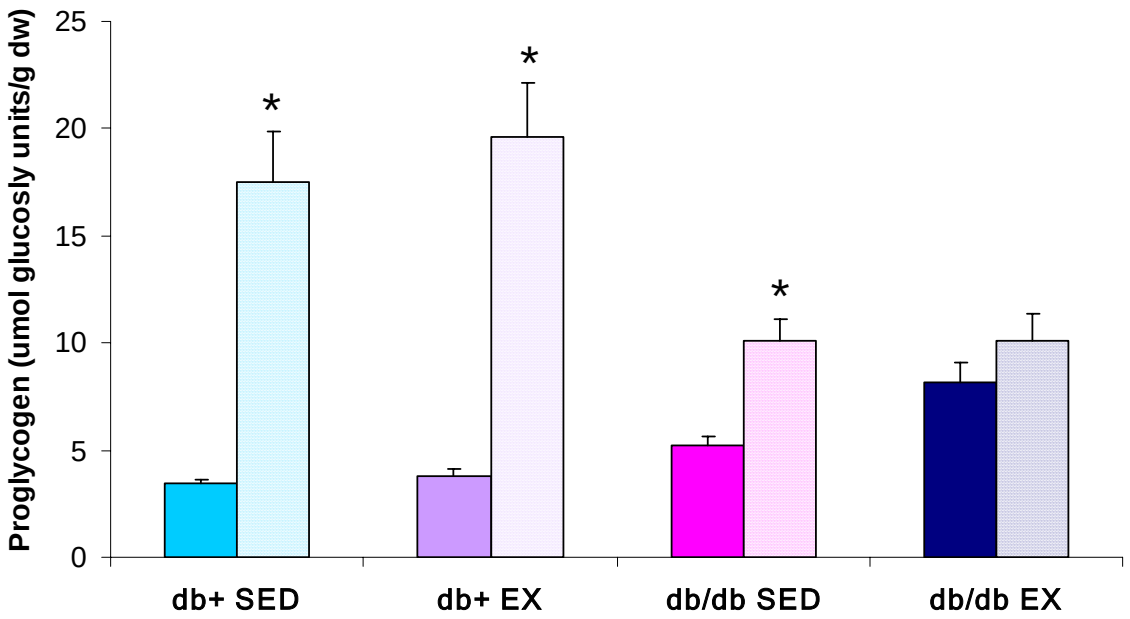

Figure 14. Proglycogen content in unperfused (solid bars) and perfused hearts (dotted bars) from non-diabetic (*db/+*) controls and C57BL/KsJ-diabetic (*db/db*) mice, with and without exercise.

*, $p<0.05$ vs. matched unperfused condition.

Unperfused

db/+ SED (N = 5); *db/+* EX (N = 5); *db/db* SED (N = 5); *db/db* EX (N = 5)

Perfused

db/+ SED (N = 20); *db/+* EX (N = 11); *db/db* SED (N = 11); *db/db* EX (N = 11)

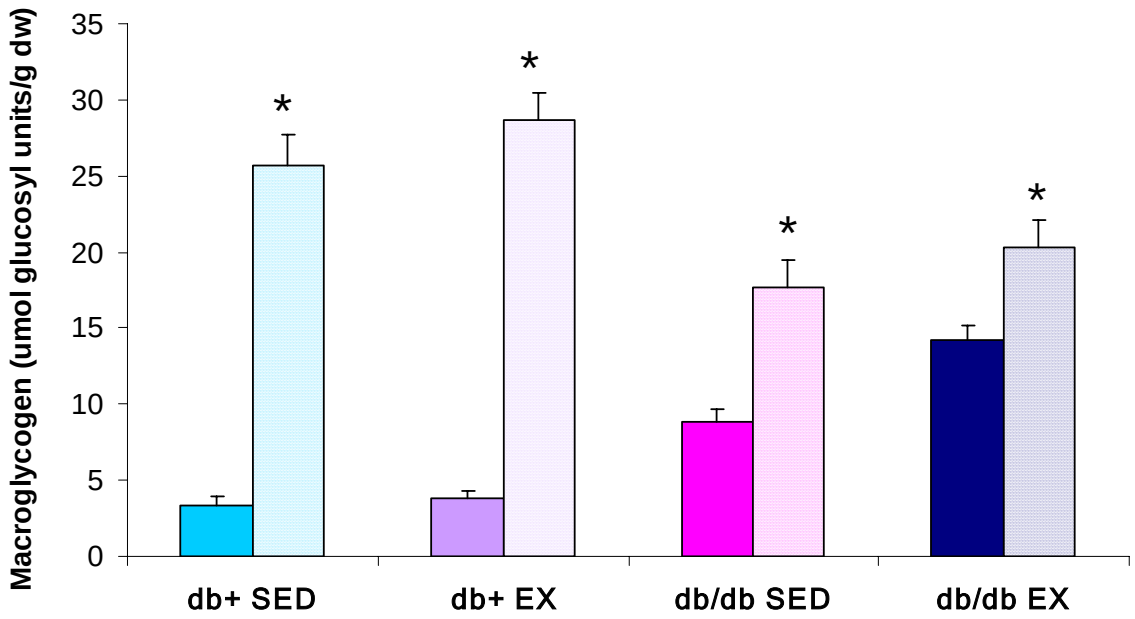

Figure 15. Macroglycogen content in unperfused (solid bars) and perfused hearts (dotted bars) from non-diabetic (*db/+*) controls and C57BL/KsJ-diabetic (*db/db*) mice, comparing trained and untrained conditions.

*, $p<0.05$ vs. matched unperfused condition.

Unperfused

db/+ SED (N = 5); *db/+* EX (N = 5); *db/db* SED (N = 5); *db/db* EX (N = 5)

Perfused

db/+ SED (N = 20); *db/+* EX (N = 11); *db/db* SED (N = 11); *db/db* EX (N = 11)

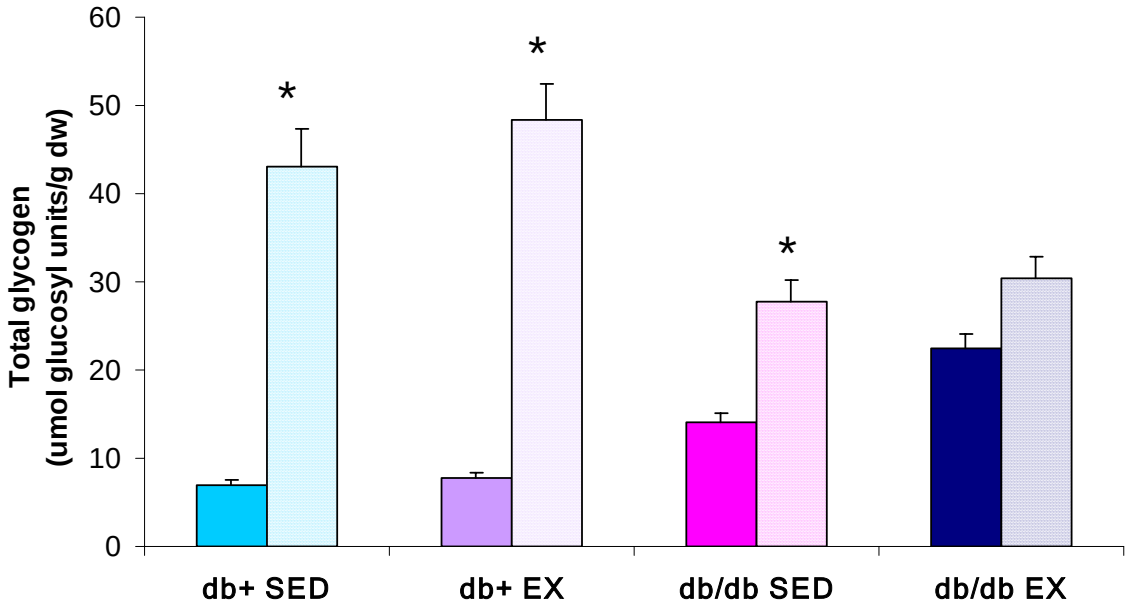

Figure 16. Total glycogen in unperfused (solid bars) and perfused (dotted bars) hearts from non-diabetic (*db/+*) controls and C57BL/KsJ-diabetic (*db/db*) mice, comparing trained and untrained conditions.

*, $p<0.05$ vs. matched unperfused condition.

Unperfused

db/+ SED (N = 5); *db/+* EX (N = 5); *db/db* SED (N = 5); *db/db* EX (N = 5)

Perfused

db/+ SED (N = 20); *db/+* EX (N = 11); *db/db* SED (N = 11); *db/db* EX (N = 11)

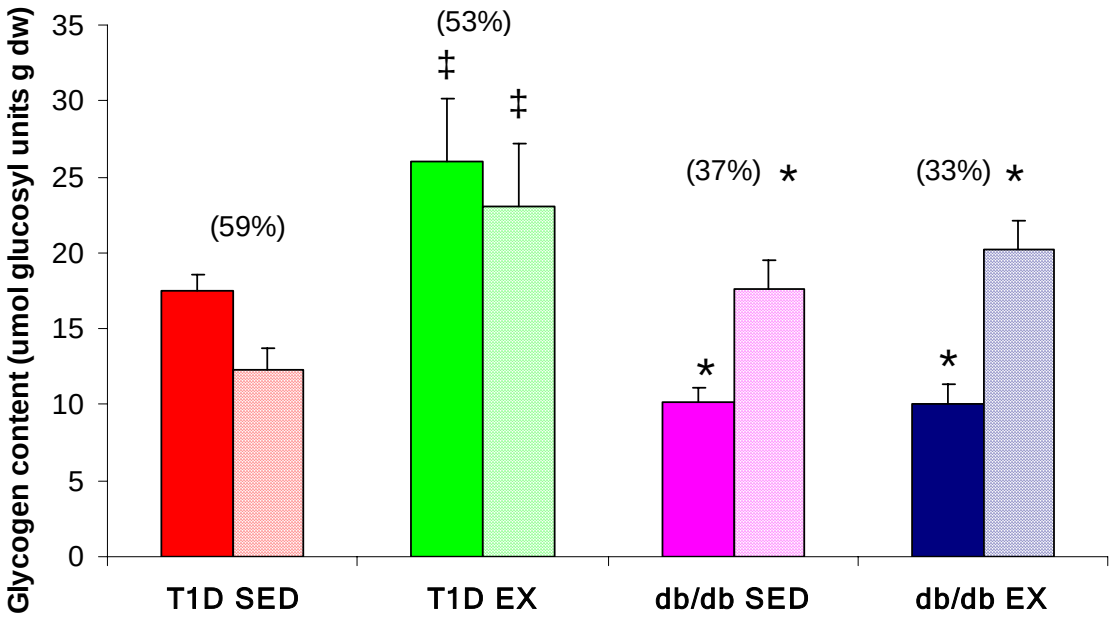

Figure 17. Proglycogen (solid bars) and macroglycogen (hatched bars) content of perfused hearts from *stz*-injected T1D and T2D (*db/db*) mice, with (EX) and without (SED) exercise. Proglycogen content as a % of total is indicated above each treatment.

*, $p<0.05$ vs. T1D hearts; ‡, $p<0.05$ vs T1D SED

T1D SED (N = 6); T1D EX (N = 6); T2D SED (N = 11); T2D EX (N = 11)

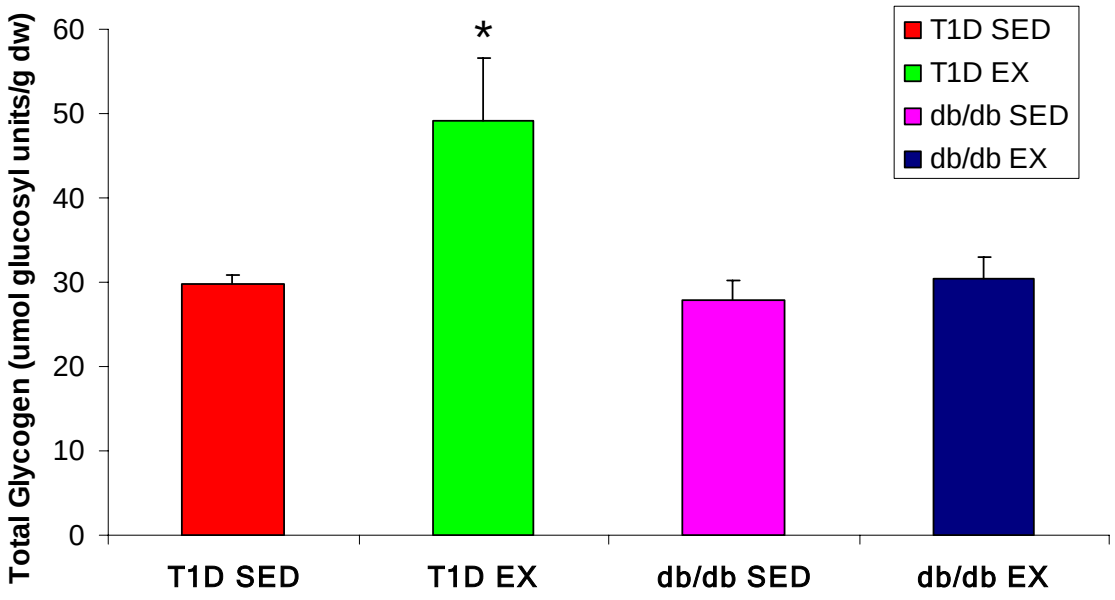

Figure 18. Total glycogen content of perfused hearts comparing *stz*-injected T1D and T2D (*db/db*) mice, with (EX) and without (SED) exercise.

*, $p<0.05$ vs. all other treatments.

T1D SED (N = 6); T1D EX (N = 6); T2D SED (N = 11); T2D EX (N = 11)

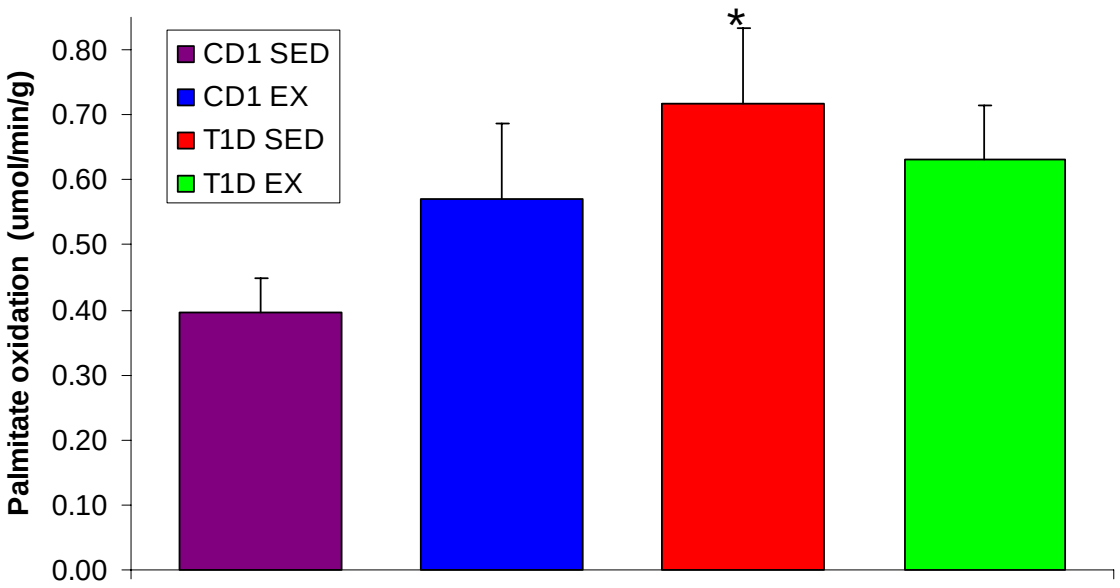

Figure 19. Palmitate oxidation rates for CD1 and *stz*-injected T1D hearts, comparing sedentary (SED) and exercised (EX) conditions.

*, $p<0.05$ vs. CD1 SED.

CD1 SED (N = 8); CD1 EX (N = 6); T1D SED (N = 6); T1D EX (N = 8).

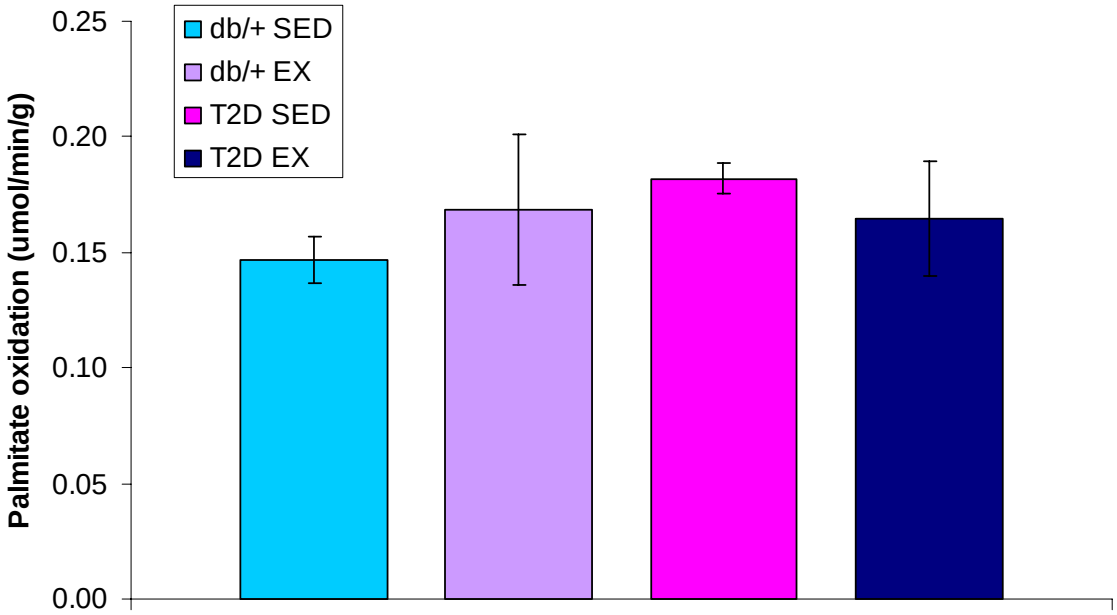

Figure 20. Palmitate oxidation rates for *db/+* and T2D (*db/db)* hearts, comparing sedentary (SED) and exercised (EX) conditions. No significant differences were revealed between groups of hearts.

db/+ SED (N = 4); *db/+* EX (N = 6); T2D SED (N = 5); T2D EX (N = 5).

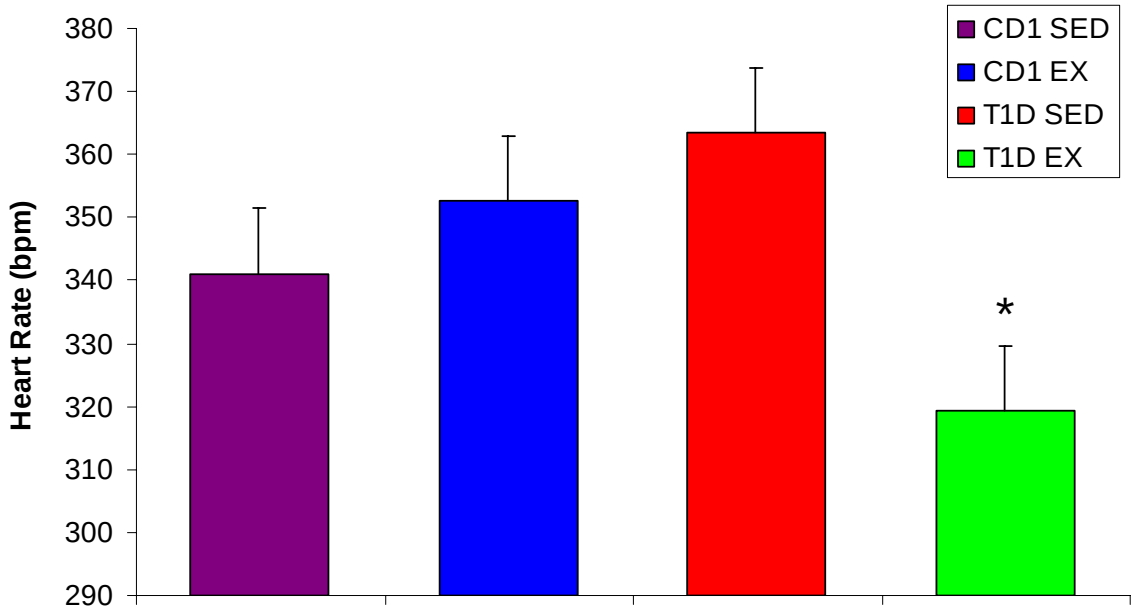

Figure 21. Mean heart rate during 1 hour perfusion comparing CD1 and *stz*-injected T1D hearts after 6 weeks exercise training (EX) and sedentary (SED) groups.

*, $p<0.05$ vs. T1D SED.

CD1 SED (N = 8); CD1 EX (N = 6); T1D SED (N = 6); T1D EX (N = 8).

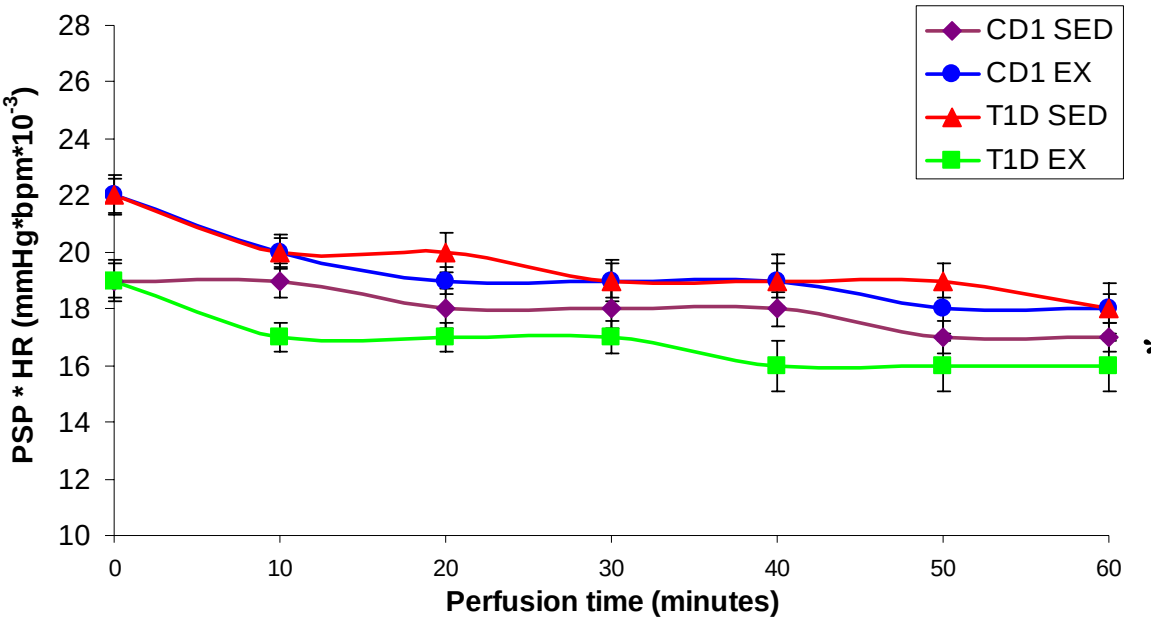

Figure 22. Rate pressure product (mmHg·bpm·10 $^{-3}$) during 1 hour perfusion for CD1 controls and *stz*-injected T1D hearts, comparing sedentary (SED) and exercised (EX) conditions.

* , $p<0.05$ vs all other treatments.

CD1 SED (N = 8); CD1 EX (N = 6); T1D SED (N = 6); T1D EX (N = 8).

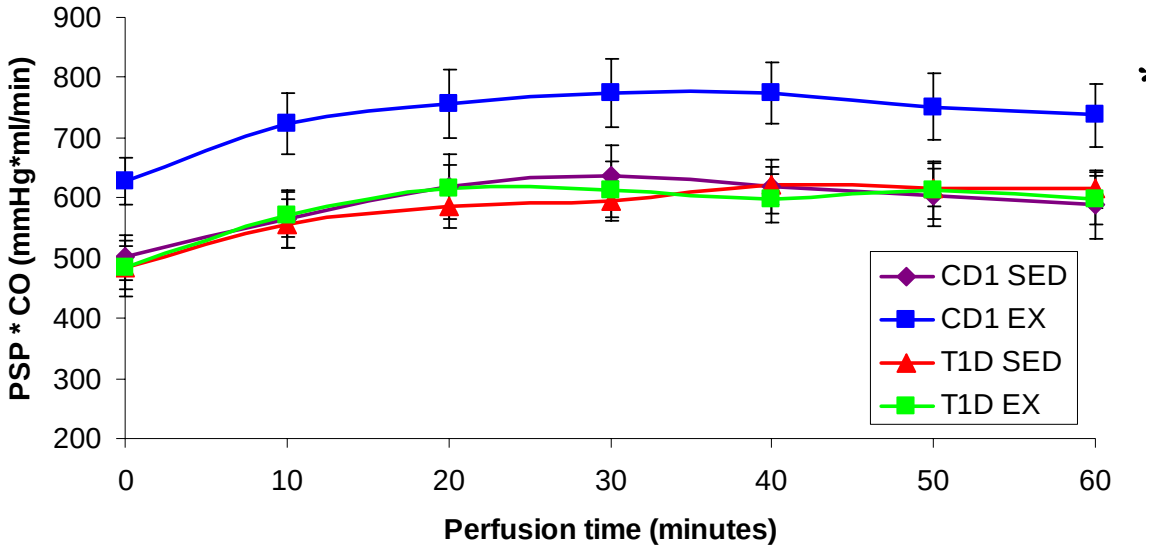

Figure 23. Hydraulic power (mmHg·ml/min) during 1 hour perfusion for CD1 controls and *stz*-injected T1D hearts, comparing sedentary (SED) and exercised (EX) conditions.

*, $p<0.05$ vs all other treatments.

CD1 SED (N = 8); CD1 EX (N = 6); T1D SED (N = 6); T1D EX (N = 8).

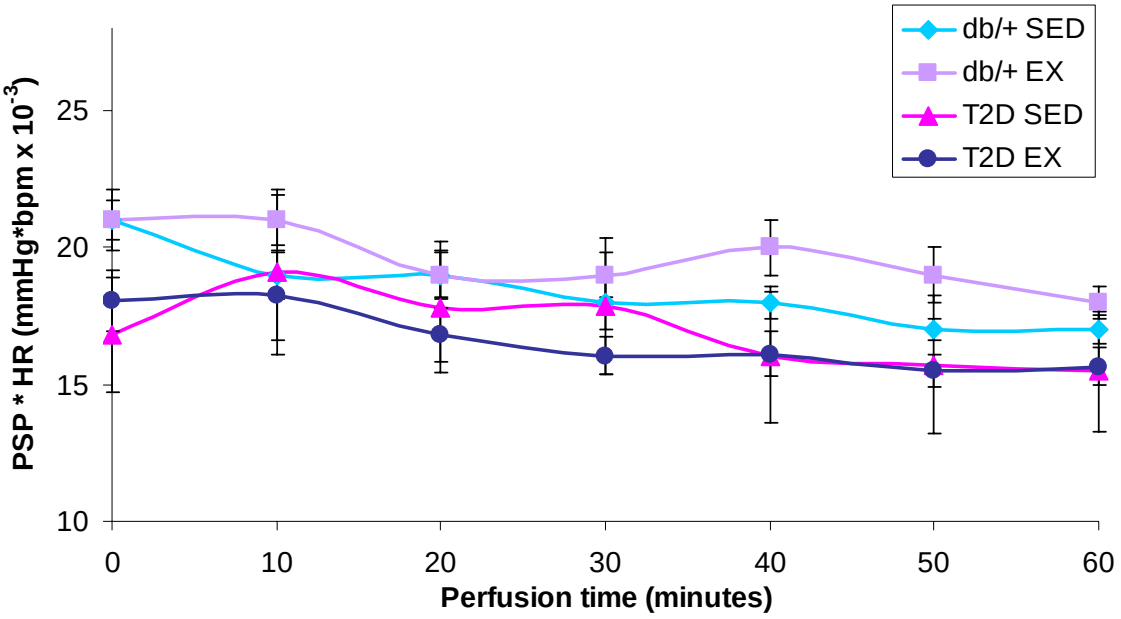

Figure 24. Rate pressure product (mmHg·bpm·10 $^{-3}$) during 1 hour perfusion time for *db/+* and T2D (*db/db)* hearts, comparing sedentary (SED) and exercised (EX) conditions. No significant differences were revealed between groups of hearts.
db/+ SED (N = 4); *db/+* EX (N = 6); T2D SED (N = 5); T2D EX (N = 5).

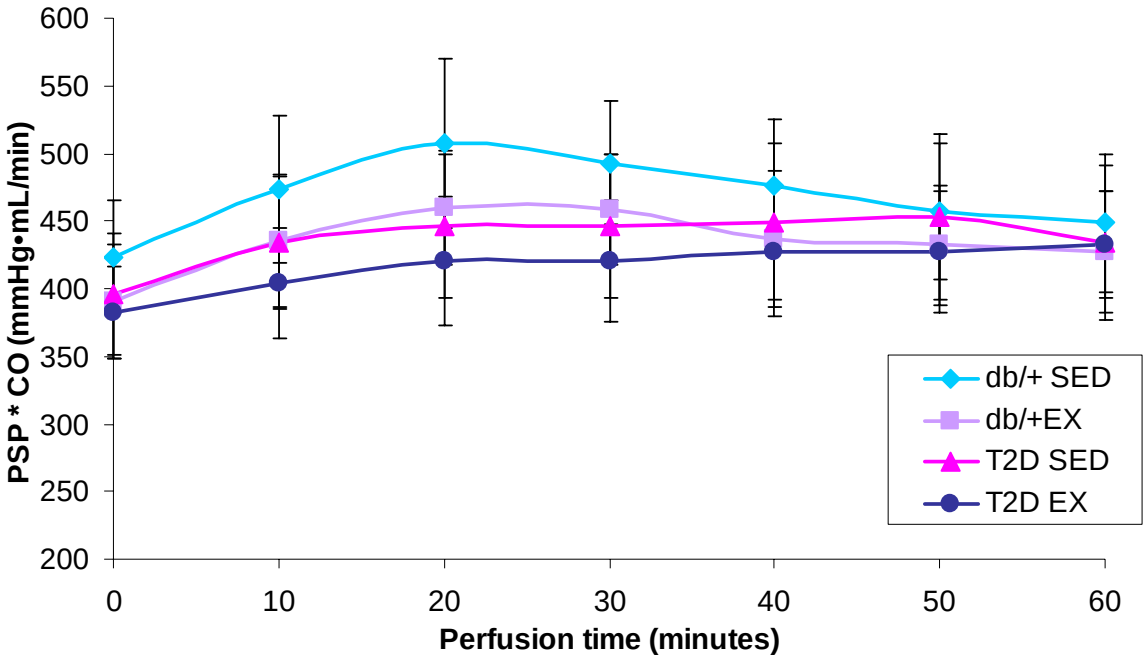

Figure 25. Hydraulic power (mmHg·mL/min) during 1 hour perfusion time for *db/+* and T2D (*db/db)* hearts, comparing sedentary (SED) and exercised (EX) conditions.
No significant differences were revealed between groups of hearts.
db/+ SED (N = 4); *db/+* EX (N = 6); T2D SED (N = 5); T2D EX (N = 5).

Table 1. Characteristics of control CD1 and *db/+* mice, T1D (*stz*-injected CD1) mice and T2D (*db/db*) mice

Group	Treatment	Pre-training body wt, g	Post training body wt, g	Blood glucose (mMol)	Heart dry wt (mg)
CD1	SED	28 ±1 (8)	40 ±1(8)	11 ±1(8)	37 ±0.1(8)
	EX	27 ±1 (8)	38 ±1 (8)	10 ±0.4 (6)	39 ±0.2 (6)
db/+	SED	25 ±0.5 (8)*	31 ±1 (8)*	12.7 ±1 (7)	30±0.5 (4)*
	EX	24 ±0.3 (8)*	29 ±1 (8)*	11.7 ±1 (8)	29±0.5 (6)*
T1D	SED	19 ±1 (8)	31 ±1 (8)	50 ±2 (6)*	25 ±0.1 (6)
	EX	21 ±1 (8)	28 ±1 (8)	41 ±3 (8)* ‡	26 ±0.1 (8)
db/db	SED	35 ±1 (8)	49 ±1 (8)	52±1 (8) **	29 ±1 (5)
	EX	35 ±1 (8)	46 ±1 (8)	44 ±2 (8) ** †	28 ±1 (5)

*, $p < 0.05$ vs CD1 SED; ‡, $p < 0.01$ vs T1D SED; **, $p<0.05$ vs *db/+* SED;
†, $p<0.05$ vs *db/db* SED

Table 2. Proglycogen, macroglycogen and total glycogen content of perfused hearts from non-diabetic *db/+* and CD1 mice, and T1D (*stz*-injected CD1), with and without exercise.

Perfused hearts	Treatment	Proglycogen (PG) (µmol/g dw)	Macroglycogen (MG) (µmol/g dw)	Total Glycogen (µmol/g dw)	PG/total (%)
db/+	SED (20)	18 ±2*	26 ±2*	43 ±4*	39 ±2
	EX (11)	20 ±3*	29 ±2*	48 ±4*	40 ±2
CD1	SED (7)	11 ±2	20 ±2	31 ±4	35 ±2
	EX (7)	9 ±1	17 ±1	27 ±1	34 ±2
T1D	SED (6)	18 ±1**	12 ±1 †	30 ±1	59 ±4**
	EX (6)	26 ±4** ‡	23 ±4 ‡	49 ±7** ‡	53 ±3**

*, $p<0.05$ *db/+* vs CD1 ; **, $p<0.05$ CD1 vs. T1D; †, $p<0.05$ T1D SED vs CD1 SED;

‡, $p<0.05$ T1D EX vs. T1D SED

Table 3. Proglycogen, macroglycogen and total glycogen content of unperfused and perfused hearts from *db/+* and T2D (*db/db*) mice comparing trained and untrained conditions.

	Treatment	Proglycogen (PG) (µmol/g dw)	Macroglycogen (MG) (µmol/g dw)	Total Glycogen (µmol/g dw)	PG/total (%)
Un-perfused hearts *db/+*	SED (5)	4 ±0.2	3 ±1	7 ±1	53 ±4
	EX (5)	4 ±0.3	4 ±0.4	8 ±1	50 ±2
T2D (*db/db*)	SED (5)	5 ±0.4	9 ±1*	14 ±1*	37 ±2*
	EX (5)	8 ±1†	14 ±1*†	22 ±2*†	36 ±1*
Perfused hearts *db/+*	SED(20)	18 ±2	26 ±2	43 ±4	39 ±2
	EX (11)	20 ±2	29 ±2	48 ±4	40 ±2
T2D (*db/db*)	SED (11)	10 ±1*	18 ±2*	28 ±2*	37 ±2
	EX (11)	10 ±1*	20 ±2*	30 ±3*	33 ±2†

*, $p<0.05$ vs. unperfused *db/+* SED; †, $p<0.05$ unperfused T2D EX vs unperfused T2D SED.

*, $p<0.05$ vs perfused *db/+* SED; †, $p<0.05$ vs perfused *db/+* SED.

Table 4. Proglycogen, macroglycogen and total glycogen content of perfused hearts from T1D and T2D mice comparing trained and untrained conditions.

Perfused hearts	Treatment	Proglycogen (PG) (μmol/g dw)	Macroglycogen (MG) (μmol/g dw)	Total Glycogen (μmol/g dw)	PG/total (%)
T1D	SED (6)	18 ±1*	12 ±1*	30 ±1*	59 ±4
	EX (6)	26 ±4	23 ±4	49 ±7	53 ±3
T2D	SED (11)	10 ±1*	18 ±1	28 ±2*	37 ±2*
	EX (11)	10 ±1*	20 ±2	30 ±3*	33 ±2*

*, $p<0.05$ vs T1D EX

Table 5. Mechanical function of isolated perfused working hearts from control CD1 and *db/+* mice, T1D (*stz*-injected CD1) mice and T2D (*db/db*) mice

Group	HR (beats/min)	Aortic flow (ml/min)	Coronary flow (ml/min)	Cardiac output (ml/min)	PSP·HR (mmHg·bpm·10^{-3})	PSP·CO (mmHg·ml·min^{-1})
CD1 SED (8)	330 ±14	8.2 ±0.7	2.6 ±0.1	10.9 ±0.6	18 ±0.6	584 ±41
CD1 EX (6)	349 ±10	10.4 ±0.7	2.9 ±0.1	13.3 ±0.8	19 ±0.7	732 ±19‡
db/+ SED(4)	338 ±12	8.0 ±0.3	1.7 ±0.1	9.7 ±0.3	18 ±0.5	468 ±11
db/+ EX (6)	359 ±20	6.4 ±0.5	1.7 ±0.2	8.1 ±0.6	20 ±0.4	434 ±9‡
T1D SED (6)	363 ±9	8.7 ±0.3	2.3 ±0.2	11.1 ±0.5	20 ±7	602 ±26
T1D EX (6)	312 ±9*	8.0 ±0.6	2.7 ±0.1	10.8 ±0.6	17 ±0.6*	585 ±32
T2D *(db/db)* SED(5)	310 ±23	6.5 ±0.8	1.6 ±0.2	8.1 ±0.9	17 ±0.5*	437 ±7
T2D *(db/db)* EX(5)	327 ±45	6.2 ±0.8	1.8 ±0.2	8.0 ±0.9	18 ±0.3*	416 ±7†

‡, $p<0.05$ vs CD1 SED
*, $p<0.05$ vs T1D SED
†, $p<0.05$ vs T2D (*db/db*) SED