EFFECT OF FREE FATTY ACIDS AND DICHLOROACETIC ACID
ON THE DIABETIC ISOLATED WORKING RAT HEART

By

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We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
August 1990
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Date Sept. 13, 1990
ABSTRACT:

It is well established that a cardiomyopathy independent of atherosclerosis develops in both humans and animals with diabetes mellitus. The etiology of diabetic cardiomyopathy is very complex involving many different processes, one of which may be the increased fatty acid utilization, and/or the concomitant decrease in glucose utilization, by the diabetic heart. We compared control and 6-week streptozotocin(STZ)-induced diabetic isolated working rat hearts and were able to demonstrate cardiac dysfunction in the diabetic as assessed by depressed heart rate (HR), heart rate peak systolic pressure product (HR X PSP), left ventricular developed pressure (LVDP), rate of pressure rise (+dP/dt) and rate of pressure decline (-dP/dt). Paralleling depressed cardiac function in the diabetic were hyperglycemia, hyperlipidemia and decreased body weight gain as compared to age-matched controls. The addition of free fatty acids, in the form of 1.2 mM palmitate, to the isolated working heart perfusate had no effect on either control or diabetic heart function, with the exception of a depressive effect on +dP/dt of diabetic hearts and -dP/dt of control hearts. But, diabetic hearts perfused with palmitate-containing perfusate plus the glucose oxidation stimulator dichloroacetate (DCA) showed a marked improvement in function. Heart rate, HR X PSP, LVDP and +/-dP/dt were all restored to control heart values in diabetic hearts perfused in the presence of DCA. Creatine phosphate and adenosine 5'-triphosphate (ATP) levels were similar under all perfusion conditions, therefore eliminating energy stores as the limiting factor in heart function. Results indicate that DCA-induced stimulation of glucose
oxidation acutely reversed diabetic cardiac function depression. Therefore, depression of glucose oxidation in the diabetic heart may be contributing to diabetic cardiomyopathy.
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To Murray, for his love, patience, moral support and encouragement whenever I needed it most

To my parents, for always being there for me
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<tr>
<td>Acetyl COA</td>
<td>Acetyl Coenzyme A</td>
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<tr>
<td>ADP</td>
<td>adenosine 5'-diphosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine 5'-triphosphate</td>
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<tr>
<td>C</td>
<td>centigrade</td>
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<tr>
<td>CAT</td>
<td>carnitine acetyltransferase</td>
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<tr>
<td>Ci</td>
<td>Curie</td>
</tr>
<tr>
<td>CoA</td>
<td>Coenzyme A</td>
</tr>
<tr>
<td>CP</td>
<td>creatine phosphate</td>
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<td>CPK</td>
<td>creatine phosphokinase</td>
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<tr>
<td>CPT</td>
<td>carnitine palmitoyl transferase</td>
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<tr>
<td>DCA</td>
<td>dichloroacetic acid</td>
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<tr>
<td>DTT</td>
<td>dithiothreitol</td>
</tr>
<tr>
<td>-dP/dt</td>
<td>rate of left ventricular pressure decline</td>
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<tr>
<td>+dP/dt</td>
<td>rate of left ventricular pressure development</td>
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<tr>
<td>EDTA</td>
<td>ethylene diamine tetraacetate</td>
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<tr>
<td>et al</td>
<td>and others</td>
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<tr>
<td>Etomoxir</td>
<td>(2-[6-(4-chlorophenoxy)hexyl]-oxirane-2-carboxylate</td>
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<tr>
<td>g</td>
<td>gram</td>
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<td>G-6-P</td>
<td>glucose-6-phosphate</td>
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<td>G-6-PDH</td>
<td>glucose-6-phosphate dehydrogenase</td>
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<td>Hepes</td>
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<td>HK</td>
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<td>HR</td>
<td>heart rate</td>
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<td>IDDM</td>
<td>insulin-dependent diabetes mellitus</td>
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i.p.  intraperitoneal
i.v.  intravenous
Kg  kilogram
KH  Krebs Henseleit
LCC  long chain acylcarnitine
LVDP  left ventricular developed pressure
ul  microliter
mg  milligram
ml  milliliter
mM  millimolar
min  minute
mw  molecular weight
N  Normal
N  sample number
NADP  nicotinamide adenine dinucleotide phosphate
NIDDM  noninsulin-dependent diabetes mellitus
nm  nanometer
nmol  nanomoles
PCA  perchloric acid
PDH  pyruvate dehydrogenase complex
POCA  2-[5-(4-chlorophenyl)penty1]-oxirane-2-carboxylate
PSP  peak systolic pressure
/  per
%  percent
rpm  revolutions per minute
S.E.M.  standard error of the mean
SR  sarcoplasmic reticulum
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<td>STZ</td>
<td>streptozocin</td>
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<td>TG</td>
<td>triglyceride</td>
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<td>U</td>
<td>units</td>
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<td>vol</td>
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<td>wt</td>
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INTRODUCTION:

Diabetes mellitus is the name given to a group of disorders characterized by fasting hyperglycemia and altered lipid metabolism, the underlying cause of which is a lack of insulin or a lack of insulin activity. This is a disease which has been recognized since antiquity. An Egyptian papyrus of 1500 BC contains a description of the symptoms. In the second century AD Aretaeus of Cappadocia named the disease diabetes, a Greek word meaning "to flow through a syphon". This graphic description implied that there was a wasting of the whole body in a copious flux of urine. Hindu manuscripts of the sixth century recognized that the urine was sweet. It was not until the eighteenth century that this sweet substance was established to be glucose, and the word mellitus (honeyed) was added.

Diabetes mellitus is now, still, a clinical disorder of major medical significance and has been reported to be the third leading cause of death in the United States, behind cardiovascular disease and cancer; the incidence in Canada is proportional. In the early 1980's there were estimated to be 10 million Americans showing overt symptoms of diabetes mellitus (Notkins, 1979). However, it has been estimated that so many cases of diabetes go unreported or undiagnosed that as many as twenty million people in the United States may be diabetic (Davidson, 1981). This number is estimated to double by the year 2000.
Diabetes mellitus can be subdivided into two major categories: insulin-dependent (IDDM) and noninsulin-dependent (NIDDM).

A lack of pancreatic insulin characterizes IDDM and results in the dependence upon daily insulin injections to control blood glucose levels, avoid ketotic coma, and remain alive. IDDM appears most commonly in youth and the etiology is complex with genetic determinants, environmental factors and immune system abnormalities included as possible factors in the genesis of the disease.

Noninsulin-dependent diabetics do not usually require insulin to maintain good control of blood glucose levels and they seldom ketotic. Usually NIDDM appears in obese adults and they are frequently hyperinsulinemic and exhibit characteristics of insulin resistance. Genetic and environmental factors play a prominent role in the etiology of NIDDM. Noninsulin-dependent diabetes is the most prevalent type of diabetes mellitus, with most of the diabetic patients in America being of the NIDDM type.

With the famous discovery of insulin in 1921 by Banting and Best, diabetes mellitus was thought to be "cured". Although the average life expectancy of a diabetic patient increased dramatically in the post-insulin era, it remains an inescapable fact that this disease is at best treatable but certainly not curable. Insulin administration has virtually eliminated coma as a cause of death in the diabetic population, but secondary disorders remain prevalent.

Some of the complications of diabetes include being twenty-five times more prone to blindness, due to retinopathy, than the normal
population. In fact, retinopathy is the leading cause of new cases of blindness. Macroangiopathy, including coronary heart disease and peripheral vascular disease, occurs prematurely and accelerates rapidly, killing seventy-five percent of non-insulin dependent diabetics. As well, ninety-three percent of diabetics of greater than twenty years duration have abnormal basement membrane thickening. Diabetics are also seventeen times more prone to kidney disease with approximately fifty percent of insulin dependent diabetics dying of chronic renal failure and twenty five percent of new renal transplant recipients being diabetic. It is also noted that fifty percent of men with long duration of diabetes are impotent and diabetics are five times more prone to gangrene, in part due to peripheral neuropathy (Podolsky, 1981).

One of the more significant secondary disorders associated with diabetes is that of heart disease, diabetics exhibit twice the incidence of heart attacks and strokes as the normal population. This increased risk is present in both insulin-dependent and noninsulin-dependent diabetics (Sievers et al, 1963). A number of clinical studies have shown that heart disease accounts for approximately eighty percent of deaths among diabetics (Kannel et al, 1974; Kannel and McGee, 1979). Mortality due to cardiovascular causes in diabetic women is five times greater than in nondiabetics (Kannel et al, 1974). Similarly, in the Rochester project, the observed cases of coronary heart disease (angina pectoris, myocardial infarction or sudden unexpected death) and of congestive heart failure without antecedent heart disease in patients with NIDDM exceeded those expected from the general population (Palumbo et al, 1981).
The pathogenesis of heart failure in diabetes is uncertain. Four major factors, namely atherosclerosis, microvascular alterations, autonomic neuropathy and primary cardiomyopathy, may be implicated in varying degrees, in concert or acting independently, in contributing to cardiovascular dysfunction during diabetes (Figure 1).

The possibility that impaired cardiac performance during diabetes may be due to a lesion in the cardiac muscle itself, independent of atherosclerotic artery disease, was raised and supported by both clinical and experimental evidence. Patients in the Framingham study who had diabetes had an exaggerated risk of clinical congestive heart failure developing compared with their nondiabetic counterparts (Kannel and McGee, 1979). This excess risk persisted even after the influence of other atherogenic risk factors and prior coronary disease status was eliminated, suggesting that factors other than coronary artery disease may be important.

Experimental evidence showed that both diabetic dogs and rats also had characteristic abnormalities in the contractile function of the heart. Regan and co-workers (1974) showed primary myocardial abnormality in alloxan-induced diabetic dogs. Following one year of experimental diabetes, dogs showed ventricular stiffening associated with shortening of left-ventricular ejection time. As well, isolated working hearts from streptozotocin- (STZ) and alloxan-induced diabetic rats showed a decreased ability to respond to increased left-atrial filling pressures
Increased Mortality Rate

Heart Failure

Defective Cardiovascular System
Increased Incidence of:
- Myocardial Infarcts
- Silent Infarcts
- Stroke
- Thrombosis
- Hypertension

Macrovascular Disease  Microvascular Disease  Primary Cardiomyopathy  Autonomic Neuropathy

Figure 1:
The sequence of events thought to ultimately cause heart failure and death in the diabetic population (Pierce et al., 1989).
(Penparagkul et al, 1980; Vadlamudi et al, 1980). Indices of cardiac function such as left-ventricular developed pressure, rates of contraction and relaxation, cardiac output and aortic output were all depressed. Cardiac function depression was also shown in diabetic rat hearts in the presence of increasing afterload pressures, resulting in decreased peak left-ventricular pressure and rate of contraction (Ingebretsen et al, 1980). Finally, isolated papillary muscles from STZ-induced diabetic rats had a depressed velocity of shortening and delayed onset of relaxation (Fein et al, 1980).

The etiology of this myocardial abnormality associated with diabetes is uncertain. One explanation comes from the observation that hearts from diabetic dogs appear to accumulate collagen which may result in ventricular stiffness and thus decreased compliance (Regan et al, 1981). However, increasing data suggest that the diabetic cardiomyopathy may be due, in part, to a combination of changes in myocardial enzyme systems and changes in subcellular organelles such sarcolemma, mitochondria, sarcoplasmic reticulum (SR), as well as contractile proteins (Dhalla et al, 1985).

Myosin and actomyosin ATPase activities have been studied during diabetes and have been found to display a diabetes-induced depression in rat hearts (Dillman, 1980; Malhotra et al, 1981; Garber et al, 1983). Similarly, myofibrillar ATPase from diabetic rat hearts displayed depressed activity (Pierce and Dhalla, 1981). This depression was suggested to be the result of a change in myosin isoenzyme distribution from the most active v1 form, found to predominate in control rats, to mainly the less active v3 form in diabetic rats (Dillman, 1980). Hence,
it can be suggested that diabetes-induced depression of myosin ATPase in the heart may lead to altered contractility.

Depression of calcium uptake into the SR has also been shown to occur in diabetic hearts (Lopaschuk et al., 1983a, 1983b). This same group proposed that a possible mechanism for this depression could be an accumulation of amphiphilic intermediates of fatty acid metabolism interfering with SR membrane function (Lopaschuk et al., 19831,2).

Defects in the sarcolemmal enzymatic activities and composition have also been observed in the diabetic rat myocardium. Altered phospholipid composition of the sarcolemma has been found and could potentially affect its function (Pierce et al., 1983). As well, a defect at the level of the basement membrane (glycocalyx) has been found in the form of altered sialic acid synthesis in sarcolemma of diabetic hearts (Pierce et al., 1983). The Na\(^{+}\)-K\(^{+}\)-ATPase enzyme responsible for maintaining the resting membrane potential of the sarcolemma has also been shown to be depressed (Pierce and Dhalla, 1981). Depression of this ion pump will obviously alter the resting membrane potential and subsequent cardiac contraction. Similarly, adenylate cyclase activity has been reported to be depressed in the diabetic heart, and therefore may be partly responsible for the reduced sensitivity to catecholamines seen in these hearts (Smith et al., 1984; Michel et al., 1985). Along with the reported decrease in alpha and beta adrenergic receptor numbers in diabetic hearts (Heyliger et al., 1982; Latifpour and McNeill, 1984), a depression in adenylate cyclase may contribute to the loss of adrenergic responsiveness of the diabetic myocardium and, hence, the normal functioning of heart. Finally, both the Na\(^{+}\)-Ca\(^{2+}\) exchanger and
Ca$^{2+}$-pump of the sarcolemma have been reported to be defective in the diabetic myocardium (Makino et al, 1987) and may, therefore also contribute to altered contractility of the diabetic heart.

Mitochondrial activity is also affected by the diabetic state, with calcium transport depression and an alteration in respiratory activity reported to occur in the mitochondria of diabetic hearts (Haugaard and Haugaard, 1964; Pierce and Dhalla, 1983). A depression in mitochondrial activity may eventually lead to a reduction in ATP levels, as reported previously, (Pieper et al, 1984; Pieper and Murray, 1987), which may result in an impairment of normal cellular function and hence, the development of diabetic cardiomyopathy.

What is apparent from the above discussion is that many detrimental and chronic changes occur in diabetic heart tissue which ultimately result in depressed cardiac function. The exact mechanism of these changes is not known but are suggested to be due to metabolic alterations at the level of the heart cell.

It is known that a prominent metabolic alteration of diabetes is an increased concentration of circulating fatty acids and concomitant increased reliance of the heart on fatty acids as an energy substrate (Morgan et al, 1961; Denton et al, 1967).

Insulin lack may be the major cause of lipid disorders of diabetes because it plays a role in both production and removal of triglyceride-rich lipoprotein. Adipose lipoprotein lipase, responsible for the removal of the triglyceride-rich lipoproteins, has been demonstrated to have decreased activity after insulin withdrawal in insulin-dependent
diabetic animals and man and to have low activity in poorly controlled diabetic subjects (Havel, 1976). An elevation of serum lipoproteins was associated with the decreased lipoprotein lipase activity. Insulin deficiency also results in a marked increase in adipose tissue lipolysis, accompanied by enhanced release of free fatty acids (FFA's) (McGarry and Foster, 1977). This occurs during diabetes because triglyceride lipase, the rate-determining enzyme in adipose tissue hydrolysis, is normally inhibited by the presence of insulin.

The clinical significance of an increase in circulating serum FFA levels is that these levels can increase the severity of a myocardial infarction (DeLeiris and Opie, 1978; Liedtke et al, 1978; Vik-Mo and Mjos, 1981) and promote and accelerate life-threatening arrhythmias (Corr et al, 1984).

Experimental evidence suggests that normalization of various aspects of fatty acid metabolism is associated with beneficial effects on the mechanical function of the diabetic heart. Diabetic rats treated with lipid-altering agents such as carnitine (Rodrigues et al, 1988), methylpalmoxirate (Tahiliani and McNeill, 1985), myoinositol (Xiang et al, 1988), and methionine and choline (Heyliger et al, 1986) displayed an improvement in cardiac performance associated with a decrease in various lipid levels. Treatment with hydralazine (Rodrigues et al, 1986) also resulted in a beneficial effect on the diabetic rat heart, again accompanied by normalization of the serum lipid profiles. These data strongly implicate altered lipid metabolism as a contributor to diabetic cardiomyopathy.
However, the exact mechanism of these effects and the degree to which they occur is not well understood. Possibly, the detrimental effects of fatty acids are due to an increased requirement for oxygen for catabolism, or to an accumulation of potentially toxic intracellular intermediates. Long chain acyl CoA and long chain acylcarnitine have been shown to accumulate in diabetic and ischemic myocardium (Idell-Wenger et al, 1978; Lopaschuk et al, 1983a, 1983b) and, as mentioned previously, have subsequently been shown to alter the function of a number of critical membrane proteins (Lopaschuk et al, 1983b; Pitts and Okhuysen, 1984; Shug et al, 1975; Adams et al, 1979).

In addition to the above discussed detrimental effects of FFA's, FFA's may have an inhibitory effect on glucose use. Under normal physiological conditions the heart utilizes about 70% fatty acids and about 30% glucose for energy production (Neely and Morgan, 1974). In the diabetic state glucose utilization is essentially abolished and fatty acids can account for up to 99% of the oxidative fuel of cardiac muscle (Randle, 1986). Recently, it was shown that glucose oxidation rates in isolated working hearts from diabetic rats were markedly depressed in the presence of physiological levels of fatty acids (Wall and Lopaschuk, 1989). An insulin lack resulting in a depression of glucose uptake is a contributor to the marked decrease in glucose utilization, but another major factor is the increase in circulating FFA's (Randle et al, 1964, 1984).

According to the concept of the glucose-fatty acid cycle, elevated serum and tissue fatty acid levels will decrease glucose oxidation. Firstly, increased citrate concentrations will inhibit
phosphofructokinase 1, with glycogenolysis and glucose uptake being subsequently decreased due to an accumulation of glucose-6-phosphate (Newsholme et al, 1962; Kerbey et al, 1985). As well, glucose oxidation is decreased in diabetes due to an inhibition of pyruvate dehydrogenase (PDH), the first irreversible reaction in the mitochondrial oxidative pathway (Garland et al, 1962). Fatty acids inhibit the PDH complex by increasing the products/substrates ratio (acetyl CoA/CoA) of the reaction. This results in a stimulation of PDH kinase which phosphorylates PDH and decreases the proportion of the complex in the the active dephosphorylated form (Randle et al, 1984). Figure 2 shows the discussed mechanisms by which fatty acids inhibit glucose use. In diabetes, the PDH complex is inhibited even further by enhanced intrinsic PDH kinase activity and increased activity of an as yet unidentified "kinase/activator" factor which is extrinsic to the complex and produces a more stable activation of PDH kinase (Kerbey et al, 1985; Randle et al, 1984).

Even though there is an increased reliance of the diabetic heart on fatty acids, it has been shown that overall rates of exogenous fatty acid oxidation are not changed (Lopaschuk and Tsang, 1987; Kreisberg, 1966; Chen et al, 1983).

The depression of glucose oxidation, in diabetes, by fatty acid metabolism has led many laboratories to try to increase glucose utilization in the diabetic heart. This has initiated the development of a number of hypoglycemic drugs which block carnitine palmitoyl transferase 1 (CPT 1), the enzyme which is responsible for catalyzing the key regulatory step in fatty acid oxidation. This CPT1 catalyzed
Figure 2:

Regulation of the entry and use of glucose residues in glycolysis. Regulatory inhibition is shown by dashed feedback arrows leading to bars across the reaction arrows. Regulatory stimulation is shown by a wide arrow parallel to the reaction arrow (adapted from Lehninger, A.L. In: Principles of Biochemistry, 1982).
reaction produces long chain acylcarnitine from cytosolic carnitine and long chain acyl CoA (Cook, 1987; Stephens et al, 1985; Tutwiler et al, 1978; Wolf et al, 1982).

Studies using a CPT1 inhibitor (POCA) in isolated perfused STZ diabetic rats demonstrated an inhibition of lipolysis and a restoration of insulin sensitivity with respect to glucose oxidation (Rosen and Reinauer, 1984). With the use of another CPT 1 inhibitor, the sylfonylurea tolbutamide, Tan et al (1984) also demonstrated increased glucose oxidation rates in isolated working hearts from chronically STZ-diabetic rats. However, these studies were carried out in the absence of exogenous fatty acids, a procedure which will in itself stimulate glucose oxidation. This is especially relevant in the chronic diabetic state which is characterized by both elevated serum glucose and FFA levels (Denton and Randle, 1967).

Recently, Wall and Lopaschuk (1989), using the CPT 1 inhibitor Etomoxir, demonstrated a significant increase in glucose oxidation rates in both the presence and absence of palmitate. They also found Etomoxir to significantly increase heart function in palmitate-perfused hearts from both control and diabetic rats. Studies have also demonstrated that Etomoxir can increase glucose utilization in tissues other than the heart (Wolf and Engel, 1985).

Etomoxir was also demonstrated to protect the diabetic heart from the adverse effects of ischemia independent of a lowering of long chain acyl carnitine or long chain acyl CoA levels (Lopaschuk and Spafford, 1989). These data suggest that accumulation of fatty acid oxidation
intermediates in the myocardium cannot account for the detrimental effects of fatty acids and diabetes in ischemic hearts nor the beneficial effect of Etomoxir. Therefore, it can be argued that impaired glucose use in diabetic rat hearts and in fatty acid perfused hearts contributes to increased susceptibility to ischemic injury.

Dichloroacetate (DCA) is another pharmacological agent which is efficacious in increasing myocardial glucose oxidation. In 1962 it was reported that DCA lowered blood and urine glucose levels and increased the respiratory quotient in diabetic rats (Lorini and Ciman, 1962). In that same year Vailati and Rabassini administered DCA to diabetic patients and found decreases in blood glucose levels. Because of this discovered potential as a hypoglycemic agent, DCA has since become the subject of many investigations.

It is now known that DCA is an inhibitor of the protein kinase involved in the regulation of the PDH complex. In 1973 Whitehouse and Randle first demonstrated that DCA activates PDH in rat hearts. The following year they found that DCA inhibits the ATP-dependent phosphorylation and inactivation of purified pig heart PDH kinase (Whitehouse and Randle, 1974). Figure 3 shows the mechanism by which DCA activates the PDH complex.

McAllister et al (1973) demonstrated that addition of millimolar concentrations of DCA to the perfusate can increase glucose oxidation rates in normal and diabetic rat hearts perfused with glucose and insulin. This was also shown to occur in hearts from normal or starved rats perfused in the presence of added acetate, ketone bodies, or
Mechanism by which dichloroacetate activates the pyruvate dehydrogenase complex (Crabb et al, 1981).
palmitate (Higgins et al, 1978). Direct addition of this agent to isolated working hearts from diabetic rats also markedly stimulates glucose oxidation; DCA was shown to increase glucose oxidation by 5000% in diabetic rat hearts perfused in the presence of FFA's (Lopaschuk and McVeigh, personal communication).

Thus, it is apparent that DCA would be a useful tool in investigating whether a depression of glucose oxidation is a contributor to diabetic cardiomyopathy by examining any acute effects this agent may have on isolated working diabetic heart function.

The purpose of this thesis was to determine if fatty acids and/or an inhibition of glucose oxidation have any detrimental effects on diabetic heart function.

The following are the major objectives of this study:

1. To attempt to more closely simulate in vivo conditions of energy substrate availability in the isolated working diabetic rat heart.

2. To investigate the effects of free fatty acids on diabetic rat heart function.

3. To investigate the effects of glucose oxidation stimulation, through the use of dichloroacetate, on a free fatty acid/glucose perfused diabetic rat heart.
MATERIALS AND METHODS

MATERIALS:

Below is a list of the chemicals, assay kits, radioisotopes, and enzymes used in this study and the companies from which they were supplied:

Averst, Montreal, Canada
halothane

BDH, Vancouver
The following chemicals were reagent grade: sodium chloride (NaCl), potassium chloride (KCl), potassium phosphate (KH$_2$PO$_4$), magnesium sulphate (MgSO$_4$-7H$_2$O), calcium chloride (CaCl$_2$-2H$_2$O), potassium hydroxide (KOH), sodium hydroxide (NaOH), perchloric acid (PCA), D-glucose, sodium hydrogen carbonate (NaHCO$_3$), Hepes, diethyl ether, chloroform, methanol, Spectrapor membrane tubing, Whatman GF/C filters

Boehringer Mannheim, Montreal
glucose assay kit, acetyl coenzyme A, nicotinamide adenine dinucleotide phosphate (NADP), hexokinase (HK), creatine phosphokinase (CPK), glucose-6-phosphate dehydrogenase (G-6-PDH), adenosine 5'-diphosphate (ADP), ethylenediamine-tetraacetic acid (EDTA)

Canada Packers Chemical, Ontario, Canada
Euthanyl - sodium pentobarbital
New England Nuclear

$^3$H Acetyl Coenzyme A, Aquasol liquid scintillant

Sigma Chemical Co., St. Louis, MO

streptozotocin (STZ), dichloroacetic acid (DCA), palmitate - free acid, bovine serum albumin (BSA), sodium carbonate ($\text{Na}_2\text{CO}_3$), carnitine acetyl transferase (CAT), sodium tetrathionate, Dowex 1-x10 anion exchange resin (7 x 12 mm)

Wako Pure Chemical Industries, Osaka, Japan

serum FFA assay kit, triglyceride assay kit
METHODS:

Animals:

Male Wistar rats, weighing between 225-275 grams, were transiently anesthetized with diethyl ether or halothane to allow injection of either streptozotocin (STZ) or its vehicle into the tail vein. Diabetes was induced by a single tail vein injection of 60 mg/kg STZ dissolved in 0.9% saline. STZ was freshly prepared before injection and was kept on ice. Control animals were injected with appropriate volumes of saline alone. Animals were provided food and water ad libitum for 6 weeks. Production of the diabetic state was diagnosed 48 hours after injection by a positive test for glycosuria with Lilly Tes-tape. Blood samples were collected at the time of killing, allowed to clot, centrifuged and the serum was analyzed for serum glucose and triglycerides (TG). STZ animals having serum glucose above 16 mmoles/L were used in this study.

Working Heart Apparatus:

The working heart perfusion apparatus used in this study was a recirculating type, a modified version of Neely's original working heart apparatus (Neely et al, 1967). The apparatus, as shown in Figure 4, consisted of a water-jacketed primary reservoir connected to a 12 gauge steel aortic cannula. The reservoir was 60 cms above the aortic cannula and contained Krebs Henseleit (KH) buffer aerated with 95% O₂/5% CO₂. A second jacketed reservoir/oxygenator was connected to a 20 gauge steel atrial cannula at a height of 15 cms above the cannula. The buffer level was maintained at a constant level by means of an 'over-flow' tube connected to the third jacketed reservoir which was situated underneath
the heart. The third reservoir also served to maintain the heart at a constant temperature as it sealed around the cork in which the cannulas were located, thereby containing the heart in an enclosed chamber. From the third reservoir the perfusate passed through a scinttered-glass filter and was then recirculated to the secondary oxygenator/reservoir by means of a Masterflex pump. The aortic cannula was also connected to a water-jacketed compliance chamber which, in turn, was connected to a length of tubing positioned at various heights above the aorta, in order to provide an external peripheral resistance, or hydrostatic afterload. This length of tubing was connected to the third reservoir where the perfusate was, as aforementioned, recirculated. The recirculating perfusate consisted of KH buffer in the presence of 3% bovine serum albumin (BSA), with palmitate, when used, being pre-bound to the albumin. The perfusate was maintained at a constant temperature of 37°C by means of a Hakke constant-temperature water recirculating pump which provided the water for the jacketed reservoirs and compliance chamber. The KH solution contained the following in millimolar (mM) quantities: NaCl 118.0, KCl 4.7, KH₂PO₄ 1.2, MgSO₄·7H₂O 1.2, CaCl₂·2H₂O 3.0, glucose 11.0, NaHCO₃ 25.0, and EDTA 5.0. When aerated with 95% CO₂/5% O₂ at 37°C, the buffer had a pH of 7.4.
The working heart apparatus consisted of three jacketed reservoirs (1, 2, 3). The primary reservoir (1) was connected to a 12 gauge steel aortic cannula (4) at a height of 60 cms above the cannula. A second jacketed reservoir (2) was connected to a 12 gauge steel atrial cannula (5) at a height of 15 cms above the cannula. A third jacketed reservoir (3) was situated below, and sealed around, the heart (6). An 'overflow' tube (7) maintained the perfusate at a constant level and was fed into the third reservoir. The aortic cannula was also connected to a water-jacketed compliance chamber (8) which, in turn, was connected to a length of tubing (9) positioned at various heights above the aorta in order to provide a hydrostatic afterload. The hydrostatic afterload fed into the third reservoir where perfusate then passed through a scintered-glass filter (10) and was pumped back up to the second reservoir by means of a Masterflex pump (11). The perfusate in the primary and secondary reservoirs was constantly aerated with 95% CO₂/5% O₂ (12) and all reservoirs and the compliance chamber were maintained at 37°C by means of a constant-temperature water recirculating pump (not shown here).

The heart (6) was attached, via the aorta, to the aortic cannula and initially perfused in a retrograde manner. The left atria was then cannulated and the perfusion then switched to the working heart mode. In the working heart mode, perfusate entering the left atria, from the secondary reservoir, was pumped out through the aortic cannula into the compliance chamber and then up the hydrostatic afterload tubing and into the third reservoir where it was recirculated. Aortic pressure was measured via a Statham P23 AA transducer (13) in line with the aortic cannula. Left ventricular pressure was measured via a transducer (14) in line with PE-90 tubing (15) pierced into the apex of the heart. The hearts were paced at 240 beats/min by electrical stimulus applied to both cannulas via a Grass model SD9D stimulator (not shown here).
Heart Perfuusions:

Animals were weighed and then anesthetized by injection (i.p.) with sodium pentobarbital (240 mg/kg). Hearts were excised from the anesthetized animals and cannulated as working hearts, as described previously (Lopaschuk et al., 1986). The hearts were initially perfused retrogradely, at 44 mm Hg pressure, through the aorta for 10 minutes with KH buffer, pH 7.4, gassed with 95% O₂/5% CO₂, containing 3.0 mM calcium and 11 mM glucose. During this time, the opening to the left atrium was cannulated. Hearts were then switched to the working heart model of perfusion with KH buffer containing glucose (11 mM), or glucose (11 mM) plus palmitate (1.2 mM). All perfusions were done in the presence of 3% BSA, with palmitate, when used, being pre-bound to the albumin. Binding palmitate to BSA involved dissolving palmitate with 1.4 mM sodium carbonate in a small amount of 2:1 water:ethanol and boiling until the ethanol had evaporated. This was quickly poured into warm KH, without glucose and with 3% BSA, followed by overnight dialysis using Spectrapor membrane tubing (vol/cm²: 5 ml, mw cutoff 6,000-8,000). After dialysis the buffer was brought to volume with KH and the appropriate amount of glucose and was filtered through a Whatman GF/C filter.

Hearts were perfused during initial work period at a left atrial filling pressure of 15 cm H₂O, and a hydrostatic afterload of 90 cm H₂O. Spontaneously beating hearts were analyzed for heart rate (HR) and for function at the medium workload. Function was described in terms of HR - peak systolic pressure product (HR X PSP), measured through an aortic transducer connected to a Grass model 79D polygraph. Hearts were then
paced at 240 beats/min and function was assessed at various afterload pressures. When hearts were paced function was based on left ventricular developed pressure (LVDP) and contractility (+/-dP/dt), measured by means of a transducer with attached PE-90 tubing pierced into the apex of the heart and connected to a Grass polygraph. Results were analyzed by an Apple II computer connected to the Grass polygraph through an A/D+D/A converter card.

The same procedure was carried out on control and diabetic hearts perfused in the presence of palmitate with and without 0.5 mM DCA. DCA was prepared as a 50 mM solution in H2O and was adjusted to pH 7.4 with NaOH.

At the end of the work period and at a maximal workload, where the afterload was made infinite by clamping off the tubing, hearts were frozen using Wollenberger clamps cooled to the temperature of liquid nitrogen. Hearts were then stored in a -80°C freezer.
Extraction of ATP, Creatine Phosphate, and Long Chain Carnitine from Ventricular Tissue:

Frozen ventricular tissue was powdered in a liquid nitrogen-cooled porcelain mortar and pestle. Approximately 200 mg of the powdered tissue was weighed, dried overnight in a 100°C oven and reweighed for determination of dry-to-wet weight ratio. Using this ratio, as well as the total frozen ventricular weight and the weight of the dried atrial tissue, total dry weight of the heart was determined.

Perchloric Acid Extraction:

Extraction of ATP, creatine phosphate (CP), and long chain acylcarnitine (LCC) were done as described previously (Idell-Wenger et al, 1978). These metabolites were extracted with the aid of mortar and pestle, from approximately 500 mg of frozen, powdered tissue into 2 ml of ice-cold perchloric acid (PCA) (6% wt/vol) containing 15 mM dithiothreitol (DTT). The tissue-PCA mixture was centrifuged (3500 rpm) at 4°C, and a portion (0.5 ml) of the supernatant was neutralized with 10 N KOH and used for determination of tissue levels of ATP and CP. The PCA precipitate was washed with 1.2% PCA and LCC was hydrolyzed at pH 13 (with 10 N KOH) for 60 min at 70°C in the presence of 15 mM DTT. The pH of the hydrolyzate was decreased to 1 with 70% PCA to precipitate any residual protein. After centrifugation at 4°C, the supernatant was neutralized with 10 N KOH and assayed for free carnitine released from its acyl esters.
ATP/Creatine Phosphate Assay:

Measurement of ATP and CP levels from the PCA extract was performed using a standard enzymatic assay as described previously (Bergmeyer, 1963)

The following set of reactions forms the basis of the assay:

\[
\text{Mg}^{2+} \quad \text{CP} + \text{ADP} \rightarrow \text{creatine} + \text{ATP} \\
\text{CPK} \\
\text{Mg}^{2+} \quad \text{glucose} + \text{ATP} \rightarrow \text{G-6-P} + \text{ADP} \\
\text{HK} \\
\text{G-6-P} + \text{NADP} \rightarrow \text{6-phosphogluconate} + \text{NADPH} \\
\text{G-6-PDH}
\]

the increase in optical density due to the formation of NADPH is measured.

Assay Procedure:

To 100 ul of sample, 100 ml of Hepes (50 mM), MgCl\textsubscript{2} (10 mM), EDTA (5 mM), 25 mg of glucose, 200 ul of glucose-6-phosphate dehydrogenase (G-6-PDH) (300 U/ml) and 3.8 mg of ADP was added. From this mixture a baseline reading was obtained on a Hewlett Packard 8452A spectrophotometer at 340 nm. This was followed by the addition of 10 ul of hexokinase (HK) (3 U/10 ul). Once a consistent reading for the addition of HK was obtained, creatine phosphokinase (CPK) 10 ul (10 U/assay) was added to the same reaction media.
The values for ATP and CP are expressed as nmoles/g dry weight of heart tissue. The perfusion medium contained no ATP or CP, therefore the values represent actual tissue values.

**Long Chain Carnitine Assay:**

Extracted carnitine released from its acyl esters was measured radiometrically as described by McGarry and Foster (1976).

Basically, the PCA extract of tissue was incubated with $^3$H Acetyl COA of known specific activity and carnitine acetyl transferase (CAT). The labelled acetyl carnitine formed according to the following reaction:

$$\text{CAT}$$

$$\text{L-carnitine} + \text{Acetyl}^3\text{H COA} \longrightarrow \text{Acetyl}^3\text{H L-carnitine} + \text{COASH}^+$$

and was separated from the unreacted $^3$H Acetyl COA by passing the mixture through a column of anion exchange resin and determining the isotope content of the effluent fluid. Sodium tetrathionate, a reducing agent, was added in order to prevent the reverse reaction from occurring.

**Assay Procedure:**

The basic reaction mixture contained, in a volume of 1.2 ml, 120 nmoles of Hepes buffer, pH 7.3, 2 umol of sodium tetrathionate, 1 unit of carnitine acetyl transferase, 25 nmol (0.07 u Ci) of $^3$H Acetyl COA, and 200 ul of sample. The reaction was initiated by addition of 1.0 ml of reaction mixture to an ependorf tube containing the sample. After
standing at room temperature for 30 min, 0.5 ml of the reaction mixture was transferred to a column of Dowex 1-x10 anion exchange resin (7 x 12 mm) and washed three times with 0.5 ml of water. The combined effluent containing $^{14}$C acetylcarnitine was collected in scintillation vials, mixed with 5 ml of aqueous counting scintillant and assayed for radioactivity in a liquid scintillation counter (Tri-Carb 4530, United Technologies Packard)

Extraction of Triglycerides from Ventricular Tissue

Tissue triglycerides were extracted as described by Bowyer and King (1977). A known weight of frozen, powdered tissue (approximately 200 mg) was extracted in 18 ml of ice-cold chloroform and methanol (2:1 vol/vol) using a Polytron homogenizer. After the addition of 3 ml of methanol, the mixture was stirred and centrifuged at 3444 g for 15 min at 4°C. The supernatant was evaporated to dryness under nitrogen, in a water bath set at 55°C.

Triglyceride Assay:

The method used to measure triglycerides was a glycerol-3-phosphate oxidase-p-chlorophenol (GPO) colouring method. This was accomplished using a test kit purchased from Wako Pure Chemical Industries, Japan.
Triglycerides are decomposed to glycerol and free fatty acids by lipoprotein lipase. The glycerol thus produced is converted to glycerol-3-phosphate by glycerol kinase in the presence of ATP. This glycerol-3-phosphate is then oxidized by GPO to yield hydrogen peroxide.

The hydrogen peroxide thus produced yields a red colour compound upon oxidative condensation with p-chlorophenol and 4-aminoantipyrine in the presence of peroxidase.

The amount of triglyceride in the original extract sample is then determined by measuring the absorbance of the developed colour, at a wavelength of 492 nm, as compared with absorbance of the standard solution.

**Assay Procedure:**

The tissue triglyceride extracts were dissolved in one mL of chloroform and mixed well. Two 10 uL samples were placed in two 12x75 mL test tubes and the chloroform was evaporated off. Then, 0.3 mL of colour reagent was added to each tube and they were incubated at 37°C for 5-10 minutes in a water bath. Following incubation, 200 uL of each mixture was placed on a Microtiter\textsuperscript{R} plate. The Microtiter\textsuperscript{R} plate was read at 492 nm on a SLT-Lab Instruments Easy Reader EAR340AT eliza reader with a Kinetic Software package.
Serum Analyses:

Serum collected at the time of sacrifice was stored at -20°C until assayed. Serum glucose was determined using a Boehringer Mannheim diagnostic kit. The method is an enzymatic colorimetric one where glucose is converted to gluconate in the presence of glucose oxidase. Hydrogen peroxide, in the presence of the enzyme peroxidase, oxidized the colourless reagent, 4-aminophenazene, to a dye whose colour development was read at 510 nm with a Brinkmann model PC800 colorimeter. Serum glucose values are expressed as mmoles/L. STZ animals having a serum glucose above 16 mmole/L were used in this study.

Serum free fatty acids were determined using a Wako Pure Chemical Industries NEFA test kit which utilizes an in vitro enzymatic colorimetric method.

The Wako enzymatic method relies upon the acylation of Coenzyme A (CoA) by the fatty acids in the presence of added Acyl CoA Synthetase. The Acyl CoA thus produced is oxidized by added Acyl CoA Oxidase with generation of hydrogen peroxide. Hydrogen peroxide, in the presence of peroxidase, permits the oxidative condensation of 3-methyl-N-ethyl-N-β-hydroxyethyl-aniline with 4-aminoantipyrine to form a purple coloured adduct which can be measured colourimetrically at 550 nm using a SLT-Lab Instruments Easy Reader EAR340AT eliza reader with a Kinetic Software package.
Statistical Analysis:

Results are presented as mean ± S.E.M. (standard error of mean). Statistical analysis was performed using a one-way ANOVA followed by a comparison of group means with a Newman-Keuls test. For heart function curves, repeated measures of analysis of variance was used, followed by a Newman-Keuls test for comparison of group means at individual points on the curve. Where only two group means were compared statistical analysis was performed using an unpaired Student's t-test. A probability of p<0.05 was taken as the level of statistical significance.
RESULTS

The Streptozotocin Diabetic Rat Model

Diabetes was induced by a single i.v. injection of 60 mg/kg STZ. Urine glucose was qualitatively assessed 48 hours after STZ injection using a Lilly Tes-tape. On a scale of 1+ (0.1%) to 4+ (2.0%), it was found to be between 3+ and 4+ for STZ-injected rats. No detectable glucose was present in the urine of control rats. In six weeks time, at the time of sacrifice, blood was collected and serum glucose levels were measured. Serum glucose levels of diabetic animals were found to be significantly higher than those of age-matched controls (Figure 5). Serum triglyceride levels were also significantly elevated in diabetic rats compared with controls (Figure 6).

Rats treated with STZ exhibited symptoms characteristic of the diabetic state. Diabetic rats gained less weight than age-matched controls and, therefore, had a significantly lower body weight at the time of sacrifice (Figure 7). Diabetics also had a noticeable increase in urine and fecal output which was not measured quantitatively.

Effect of Free Fatty Acids on Control and Diabetic Isolated Working Rat Hearts

To investigate the effects of FFA's on diabetic heart function, isolated working rat hearts were perfused in the presence of glucose (11 mM) alone or glucose (11 mM) plus palmitate (1.2 mM). In spontaneously
beating hearts, at a medium workload (15 cm H₂O filling pressure, 90 cm H₂O afterload pressure), heart rate was found to be significantly lower in diabetic hearts compared with controls (Figure 8). Figure 8 also shows that FFA's had no significant effect on heart rate.

HR X PSP was measured at a medium workload and was found to be depressed in diabetics, regardless of available substrates (Figure 9). This figure also shows that, in both controls and diabetics, the presence of FFA's had no significant effect on HR X PSP.

Figure 10 shows LVDP, measured at a medium workload, to be significantly depressed in diabetic hearts compared with control hearts. The presence of FFA's had no significant effect on LVDP in either group of hearts. Similarly, maximal LVDP, measured at an infinite afterload pressure, was depressed in diabetic hearts compared with controls (Figure 11). The presence of FFA's had no significant effect on maximal LVDP in either control or diabetic hearts.

LVDP was also measured at five different afterload pressures (60, 70, 90, 140 cm H₂O, and maximal) and was found to be significantly depressed, at each afterload pressure, in both diabetic groups with respect to controls (Figure 12). Again, the presence of FFA's had no significant effect.

Positive dP/dt was measured at five different afterload pressures and was found to be depressed, at each afterload pressure, in diabetics perfused in the presence of FFA's compared with controls perfused in either condition (Figure 13). Positive dP/dt was also found to be depressed in diabetics perfused in the presence of FFA's compared with
diabetics perfused in the presence of glucose alone. This depression was found at each afterload pressure with the exception of 140 cm H2O. Only at an infinite afterload pressure did diabetic hearts perfused in the presence of glucose alone have a +dP/dt significantly depressed from controls perfused under either condition. FFA's had no detrimental effect on +dP/dt in control hearts.

Figure 14 demonstrates -dP/dt measured at five different afterload pressures. Both diabetic groups had significantly depressed -dP/dt's, at all afterload pressures, with respect to both control groups. There was an exception, where at a 70 cm H2O afterload, diabetics perfused in the presence of glucose alone were only significantly different from controls perfused in the presence of glucose alone. FFA's had no detrimental effect on diabetic -dP/dt but had an effect on control hearts. At afterload pressures of 60, 70, 90 and 140 cm H2O controls, perfused in the presence of FFA's, had significantly depressed -dP/dt's compared with controls perfused in the presence of glucose alone.

Effect of DCA on FFA Perfused Control and Diabetic Isolated Working Rat Hearts

As described in the introduction, DCA is a PDH activator which results in increased glucose oxidation rates when added to isolated working heart perfusate.

Figure 15 demonstrates that, at a medium workload, the addition of 0.5 mM DCA to the FFA perfusate resulted in a significantly higher HR in
diabetic hearts than in diabetic hearts perfused in the presence of 
FFA’s alone. The diabetic hearts perfused in the presence of DCA showed 
similar HR’s to those of controls.

HR X PSP, measured at a medium workload, was also found to be 
significantly depressed in diabetics compared with controls and with 
diabetics perfused in the presence of 0.5 mM DCA (Figure 16).

Figure 17 shows LVDP, measured at a medium workload, to be 
significantly decreased in diabetics with respect to diabetics perfused 
in the presence of 0.5 mM DCA and with respect to controls. Likewise, 
maximal LVDP, demonstrated in Figure 18, was found to be significantly 
lower in diabetic hearts compared with controls or with diabetic hearts 
perfused in the presence of 0.5 mM DCA.

Measured at five different afterload pressures, LVDP was found to 
be significantly lower in diabetic hearts, at 70, 90 cm H₂O and maximal 
afterload pressures, with respect to controls and with respect to 
diabetics perfused in the presence of 0.5 mM DCA (Figure 19). At a 60 
cm H₂O afterload diabetic hearts perfused in the absence of DCA had a 
significantly lower LVDP than control hearts only.

Positive dP/dt was measured at five different afterload pressures 
and was found to be significantly depressed at each afterload pressure 
compared with controls and with diabetics perfused in the presence of 
0.5 mM DCA (Figure 20).

Figure 21 demonstrates -dP/dt, measured at five different 
afterload pressures, to be significantly lower in diabetic hearts, at
90, 140 cm $H_2O$ and maximal afterloads, compared with control hearts and compared with diabetic hearts perfused in the presence of 0.5 mM DCA. At 60 and 70 cm $H_2O$ afterloads, diabetic hearts perfused in the absence of DCA had a significantly depressed $-dP/dt$ compared with control hearts only.

**Effect of Free Fatty Acids on Ventricular Tissue Metabolites in Isolated Working Control and Diabetic Hearts**

Following the aforementioned isolated working heart perfusion protocol, and at an infinite afterload, hearts were immediately frozen with Wollenberger clamps cooled to the temperature of liquid nitrogen. Tissue metabolites were extracted and assayed from the ventricular tissue, as described in the methods section.

Figure 22 demonstrates that ATP (umoles/g dry wt) was not significantly different in diabetic hearts compared with control hearts. This figure also shows that the presence of FFA’s in the working heart perfusate had no effect on the levels of ATP found in either control or diabetic hearts.

It was found that creatine phosphate levels were not significantly different in diabetic hearts compared with control hearts (Figure 23). It was also shown that the presence of FFA’s in the working heart perfusate had no effect on creatine phosphate levels in either control or diabetic hearts.
Figure 24 demonstrates that ventricular tissue LCC levels were not significantly different in diabetics with respect to controls. This figure also shows that the presence of FFA's in the working heart perfusate had no effect on LCC levels in either control or diabetic hearts.

Figure 25 shows that tissue triglyceride levels were not changed by either the presence of diabetes or by the use of FFA's in the working heart perfusate.

Effect of DCA on Ventricular Tissue Metabolite Levels in Free Fatty Acid Perfused Control and Diabetic Hearts

Figure 26 demonstrates that levels of ATP in ventricular tissue were not significantly affected by the presence of diabetes or by the presence of 0.5 mM DCA in the FFA working heart perfusate.

No significant difference was found for creatine phosphate levels of ventricular tissue in diabetics compared with controls, or in diabetics or controls perfused in the presence of 0.5 mM DCA (Figure 27).

Figure 28 shows ventricular LCC levels to be similar in control and diabetic ventricular tissue regardless of the presence of 0.5 mM DCA in the working heart perfusate.

Ventricular tissue triglycerides levels are demonstrated in Figure 29. There was a trend toward levels being increased in diabetic hearts.
perfused in the presence of 0.5 mM DCA although the increase was not statistically significant.

Rationale for the Use of Afterload Curves

Common throughout the literature are examinations of cardiac function through comparison of performances of isolated working hearts in response to changes in filling pressure (Rodrigues et al, 1988; Tahiliani et al, 1985; Vadlamudi et al, 1982; Xiang et al, 1988). It has been well established that LVDP and +/-dP/dt increase, at different rates in controls and diabetics, in response to increases in filling pressure (Vadlamudi et al, 1982). However, we found very little, or no response, by way of LVDP or +/-dP/dt, to changes in filling pressure. Rather, we found a marked response to changes in hydrostatic afterload pressure and therefore, used afterload curves throughout our study as opposed to preload, or filling pressure curves.

Upon further investigation it became apparent that the major functional difference between the methodology used by us and that used by previous workers was a difference in the kind of afterload system used. This was found to be the basis for the difference in response to changes in filling pressure.

The previous researchers had utilized a fixed resistance-type afterload whereas our studies utilized a hydrostatic type afterload. It is obvious that any increase in filling pressure equals an increase in flow rate through the afterload device. An increase in flow through a
hydrostatic afterload will have no effect on the afterload pressure the heart experiences. However, as shown in Figure 30 and Table 1, an increase in flow rate through a resistance-type afterload results in an increase in pressure, or an increase in effective afterload the heart experiences. Therefore, it can be assumed that previous workers showing changes in LVDP and +/-dP/dt in response to changes in filling pressure were actually demonstrating a combined effect of both preload and afterload. For this reason, we chose to compare control and diabetic isolated working rat heart function through the comparison of responses to changes in afterload pressure.
Figure 5:

Serum glucose levels of diabetic rats 6 weeks after induction of diabetes with STZ and of age-matched controls.

Results are expressed as mean ± S.E.M. (* denotes values which are significantly different from controls at p<0.05).
Serum Glucose Concentration Of Six Week Control And Diabetic Rats

* - significantly different from control
Figure 6:

Serum free fatty acid levels of diabetic rats 6 weeks after induction of diabetes with STZ and of age-matched controls.

Results are expressed as mean ± S.E.M. (* denotes values which are significantly different from controls at p<0.05)
Serum Free Fatty Acid Concentration Of Six Week Control And Diabetic Rats

* - significantly different from control

N = 16

N = 23
Figure 7:

Body weight change of diabetic and control rats six weeks following injection of STZ or its vehicle.

Results are expressed as mean ± S.E.M. (* denotes values which are significantly different from controls at p<0.05).
Body Weight Change Over A Six Week Period Of Control And Diabetic Rats

- Diabetic
- Control

Body Weight Change (gms)

* - significantly different from control

N = 23
Figure 8:

Effect of metabolic substrates on heart rate in control and diabetic isolated working rat hearts at a medium workload (90 cm H₂O afterload).

Results are expressed as mean ± S.E.M. (* denotes values which are significantly different from both control groups at p<0.05).
EFFECT OF METABOLIC SUBSTRATES ON HEART RATE IN CONTROL AND DIABETIC ISOLATED WORKING RAT HEARTS AT A MEDIUM WORKLOAD (90 cm H2O AFTERLOAD)

N=7  N=8  N=7  N=8

HEART RATE (beats/min.)

Control Glucose  Diabetic Glucose
Control Palmitate  Diabetic Palmitate

* = significantly different from both control groups
Figure 9:

Effect of metabolic substrates on HR X PSP in control and diabetic isolated working rat hearts at a medium workload (90 cm H₂O afterload).

Results are expressed as mean ± S.E.M. (* denotes values which are significantly different from both control groups at p<0.05).
EFFECT OF METABOLIC SUBSTRATES ON HR X PSP IN CONTROL AND DIABETIC ISOLATED WORKING RAT HEARTS AT A MEDIUM WORKLOAD (90 cm H2O AFTERLOAD)

EFFECT OF METABOLIC SUBSTRATES ON HR X PSP IN CONTROL AND DIABETIC ISOLATED WORKING RAT HEARTS AT A MEDIUM WORKLOAD (90 cm H2O AFTERLOAD)

N=7  N=7

N=7  N=8

* = significantly different from both control groups
Figure 10:

Effect of metabolic substrates on LVDP in control and diabetic working rat hearts at a medium workload (90 cm H$_2$O afterload).

Results are expressed as mean ± S.E.M. (* denotes values which are significantly different from both control groups at p<0.05).
EFFECT OF METABOLIC SUBSTRATES ON LVDP IN CONTROL AND DIABETIC ISOLATED WORKING RAT HEARTS AT A MEDIUM WORKLOAD (90 cm H2O AFTERLOAD)

LVDP mmHg

- Control Glucose
- Control Palmitate
- Diabetic Glucose
- Diabetic Palmitate

* - significantly different from both control groups
Figure 11:

Effect of metabolic substrates on maximal LVDP in control and diabetic isolated working rat hearts.

Results are expressed as mean ± S.E.M. (* denotes values which are significantly different from both control groups at p<0.05).
EFFECT OF METABOLIC SUBSTRATES ON MAXIMUM LVDP IN CONTROL AND DIABETIC ISOLATED WORKING RAT HEARTS

![Graph showing LVDP in control and diabetic hearts with different metabolic substrates.](image)

- Control Glucose
- Diabetic Glucose
- Control Palmitate
- Diabetic Palmitate

* = significantly different from both control groups

Legend:

- Control Glucose
- Diabetic Glucose
- Control Palmitate
- Diabetic Palmitate

N=9  N=7  N=7  N=8
Figure 12:

Effect of metabolic substrates on LVDP, measured at five different afterload pressures, in control and diabetic isolated working rat hearts.

Results expressed as mean ± S.E.M. (* denotes values which are significantly different from both control groups at p<0.05).
EFFECT OF METABOLIC SUBSTRATES ON LVDP IN CONTROL AND DIABETIC ISOLATED WORKING RAT HEARTS

* - significantly different from both control groups
Figure 13:

Effect of metabolic substrates on dP/dt, measured at five different afterload pressures, in control and diabetic isolated working rat hearts.

Results expressed as mean ± S.E.M. (* denotes values which are significantly different from all other groups, + denotes values which are significantly different from Diabetic Palmitate, £ denotes values which are significantly different from Control Glucose, † denotes values which are significantly different from Control Palmitate at p<0.05).
EFFECT OF METABOLIC SUBSTRATES ON +DP/DT IN CONTROL AND DIABETIC ISOLATED WORKING RAT HEARTS

- Control Glucose
- Control Palmitate
- Diabetic Glucose
- Diabetic Palmitate

* - significantly different from all other groups
+ - significantly different from Diabetic Palmitate
Σ - significantly different from Control Glucose
† - significantly different from Control Palmitate

N = 7–9

Afterload Pressure cmH2O

MAX

2400
3400
4400
5400
6400
7400
Figure 14:

Effect of metabolic substrates on -dP/dt in control and diabetic isolated working rat hearts.

Results are expressed as mean ± S.E.M. (* denotes values which are significantly different from both control groups, + denotes values which are significantly different from Control Palmitate, £ denotes values which are significantly different from Control Glucose at p<0.05).
EFFECT OF METABOLIC SUBSTRATES ON $-\frac{dP}{dt}$ IN CONTROL AND DIABETIC ISOLATED WORKING RAT HEARTS

Graph showing the effect of different metabolic substrates on $-\frac{dP}{dt}$ in control and diabetic isolated working rat hearts. Lines represent different conditions:
- Control Glucose
- Control Palmitate
- Diabetic Glucose
- Diabetic Palmitate

Significance markers:
- * significantly different from both control groups
- + significantly different from Control Palmitate
- $\xi$ significantly different from Control Glucose

N = 7-9
Figure 15:

Effect of DCA on heart rate in FFA perfused control and diabetic isolated working rat hearts at a medium workload (90 cmH$_2$O afterload).

Results are expressed as mean ± S.E.M. (* denotes values which are significantly different from all other groups at $p<0.05$).
EFFECT of DICHLOROACETIC ACID on HEART RATE IN FATTY ACID PERFUSED CONTROL AND DIABETIC RAT HEARTS at a MEDIUM WORKLOAD (90 cmH2O AFTERLOAD)

HEART RATE (beats/min.)

- Control
- Control + .5mM DCA
- Diabetic
- Diabetic + .5mM DCA

N=8 N=6 N=7

* – significantly different from all other groups
Figure 16:

Effect of DCA on HR X PSP in FFA perfused control and diabetic isolated working rat hearts at a medium workload (90 cm H$_2$O afterload).

Results are expressed as mean ± S.E.M. (* denotes values which are significantly different from all other groups at p<0.05).
EFFECT of DICHLOROACETIC ACID on HR X PSP IN FATTY ACID PERFUSED CONTROL AND DIABETIC RAT HEARTS at a MEDIUM WORKLOAD (90 cmH2O AFTERLOAD)

![Graph showing the effect of DICHLOROACETIC ACID on HR X PSP in fatty acid perfused control and diabetic rat hearts at a medium workload.](image)

- Control
- Control + .5mM DCA
- Diabetic
- Diabetic + .5mM DCA

* - significantly different from all other groups

N = 6
Figure 17:

Effect of DCA on LVDP in FFA perfused control and diabetic isolated working rat hearts at a medium workload (90 cmH$_2$O afterload).

Results are expressed as mean ± S.E.M. (* denotes values which are significantly different from all other groups at p<0.05).
EFFECT of DICHLOROACETIC ACID on LVDP in FATTY ACID PERFUSED CONTROL AND DIABETIC RAT HEARTS at a MEDIUM WORKLOAD (90 cmH2O AFTERLOAD)

<table>
<thead>
<tr>
<th>Group</th>
<th>LVDP mmHg</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>140</td>
<td>7</td>
</tr>
<tr>
<td>Control + .5mM DCA</td>
<td>140</td>
<td>7</td>
</tr>
<tr>
<td>Diabetic</td>
<td>120</td>
<td>8</td>
</tr>
<tr>
<td>Diabetic + .5mM DCA</td>
<td>120</td>
<td>7</td>
</tr>
</tbody>
</table>

* — significantly different from all other groups
Figure 18:

Effect of DCA on maximal LVDP in FFA perfused control and diabetic rat hearts.

Results are expressed as mean ± S.E.M. (* denotes values which are significantly different from all other groups at p<0.05).
EFFECT of DICHLOROACETIC ACID on MAXIMAL LVDP in FREE FATTY ACID PERFUSED CONTROL AND DIABETIC RAT HEARTS

![Graph showing the effect of DICHLOROACETIC ACID on LVDP.](Image)

- **Control**
- **Control + .5mM DCA**
- **Diabetic**
- **Diabetic + .5mM DCA**

* - significantly different from all other groups
Figure 19:

Effect of DCA on LVDP, measured at five different afterload pressures, in FFA perfused control and diabetic isolated working rat hearts.

Results are expressed as mean ± S.E.M. (* denotes values which are significantly different from all other groups, + denotes values which are significantly different from both control groups at p<0.05).
EFFECT OF DICHLOROACETIC ACID ON LVDP IN FATTY ACID PERFUSED CONTROL AND DIABETIC ISOLATED WORKING RAT HEARTS

Control
Control + .5mM DCA
Diabetic
Diabetic + .5mM DCA

N = 6-8

* - significantly different from all other groups
+ - significantly different from both control groups
Figure 20:

Effect of DCA on \( +dP/dt \), measured at five different afterload pressures, in FFA perfused control and diabetic isolated working rat hearts.

Results are expressed as mean ± S.E.M. (* denotes values which are significantly different from all other groups at \( p<0.05 \)).
EFFECT OF DICHLOROACETIC ACID ON $+\frac{dP}{dT}$ IN FATTY ACID PERFUSED CONTROL AND DIABETIC ISOLATED WORKING RAT HEARTS

- 71

Afterload Pressure cmH20

Control
Control + .5mM DCA
Diabetic
Diabetic + .5mM DCA

$+\frac{dP}{dT}$

N=6–8

$*$ - significantly different from all other groups
Figure 21:

Effect of DCA on $-\frac{dP}{dt}$, measured at five different afterload pressures, in FFA perfused control and diabetic isolated working rat hearts.

Results are expressed as mean ± S.E.M. (* denotes values which are significantly different from all other groups, † denotes values which are significantly different from both control groups at $p<0.05$).
EFFECT OF DICHLOROACETIC ACID ON $-\frac{\text{DP}}{\text{dT}}$ IN FATTY ACID PERFUSED CONTROL AND DIABETIC ISOLATED WORKING RAT HEARTS

![Graph](image-url)

- **Control**
- **Control + .5mM DCA**
- **Diabetic**
- **Diabetic + .5mM DCA**

* - significantly different from all other groups
† - significantly different from both control groups

**N = 6 – 8**
Figure 22:

Effect of metabolic substrates on tissue ATP in control and diabetic isolated working hearts at a maximal workload.

Results are expressed as mean ± S.E.M.
EFFECT OF METABOLIC SUBSTRATES ON TISSUE ATP IN CONTROL AND DIABETIC ISOLATED WORKING HEARTS AT A MAXIMAL WORKLOAD

N=7  N=5  N=7  N=7

ATP umoles/g dry wt.

- Control Glucose
- Diabetic Glucose
- Control Palmitate
- Diabetic Palmitate
Figure 23:

Effect of metabolic substrates on tissue creatine phosphate in control and diabetic isolated working hearts at a maximal workload.

Results are expressed as mean ± S.E.M.
EFFECT OF METABOLIC SUBSTRATES ON TISSUE CREATINE PHOSPHATE IN CONTROL AND DIABETIC ISOLATED WORKING HEARTS AT A MAXIMAL WORKLOAD

![Graph showing the effect of metabolic substrates on tissue creatine phosphate in control and diabetic isolated working hearts at a maximal workload.](image-url)
Figure 24:

Effect of metabolic substrates on tissue LCC's in control and diabetic isolated working hearts at a maximal workload.

Results are expressed as mean ± S.E.M.
EFFECT OF METABOLIC SUBSTRATES ON TISSUE LCC'S IN CONTROL AND DIABETIC ISOLATED WORKING HEARTS AT A MAXIMAL WORKLOAD

![Graph showing the effect of metabolic substrates on tissue LCC's in control and diabetic isolated working hearts at a maximal workload. The graph includes bars for control and diabetic glucose, control and diabetic palmitate with different sample sizes (N=5, N=7).]
Figure 25:

Effect of metabolic substrates on tissue triglycerides in control and diabetic isolated working hearts at a maximal workload.

Results are expressed as mean ± S.E.M.
EFFECT OF METABOLIC SUBSTRATES ON TISSUE TRIGLYCERIDES IN CONTROL AND DIABETIC ISOLATED WORKING HEARTS AT A MAXIMAL WORKLOAD

![Graph showing the effect of metabolic substrates on tissue triglycerides in control and diabetic isolated working hearts at a maximal workload. The graph compares glucose and palmitate in control and diabetic conditions, with different sample sizes (N=9, N=6, N=7, N=8) for each condition.]
Figure 26:

Effect of DCA on tissue ATP in FFA perfused control and diabetic isolated working rat hearts at a maximal workload.

Results are expressed as mean ± S.E.M.
EFFECT of DICHLOROACETIC ACID on TISSUE ATP IN FATTY ACID PERFUSED CONTROL AND DIABETIC ISOLATED WORKING RAT HEARTS AT A MAXIMAL WORKLOAD

ATP umoles/g dry wt.

N=5  N=8  N=7  N=5

Control  Control + .5mM DCA  Diabetic  Diabetic + .5mM DCA
Figure 27:

Effect of DCA on tissue creatine phosphate in FFA perfused control and diabetic isolated working rat hearts at a maximal workload.

Results are expressed as mean ± S.E.M.
EFFECT of DICHLOROACETIC ACID on TISSUE CREATINE PHOSPHATE IN FATTY ACID PERFUSED CONTROL AND DIABETIC ISOLATED WORKING RAT HEARTS AT A MAXIMAL WORKLOAD.
Figure 28:

Effect of DCA on tissue LCC's in FFA perfused control and diabetic isolated working rat hearts at a maximal workload.

Results are expressed as mean ± S.E.M.
EFFECT OF DICHLOROACETIC ACID ON TISSUE LCC'S IN FATTY ACID PERFUSED CONTROL AND DIABETIC ISOLATED WORKING RAT HEARTS AT A MAXIMAL WORKLOAD
Figure 29:

Effect of DCA on tissue triglycerides in FFA perfused control and diabetic isolated working rat hearts at a maximal workload.

Results are expressed as mean ± S.E.M.
EFFECT of DICHLOROACETIC ACID on TISSUE TRIGLYCERIDES IN FATTY ACID PERFUSED CONTROL AND DIABETIC ISOLATED WORKING RAT HEARTS AT A MAXIMAL WORKLOAD

![Graph showing the effect of dichloroacetic acid on tissue triglycerides in fatty acid perfused control and diabetic isolated working rat hearts at a maximal workload.](image-url)
Figure 30:

Pressure produced by increased flow rate through a resistance-type afterload system.
PRESSURE PRODUCED BY INCREASED FLOW RATE THROUGH A RESISTANCE-TUBING TYPE AFTERLOAD SYSTEM
Table 1:

Pressure produced by increased flow rate through a resistance-type afterload system.
Pressure Produced by Increased flow Rate Through a Resistance-tubing Type Afterload System

<table>
<thead>
<tr>
<th>Flow rate (mL/min)</th>
<th>Pressure (mm Hg)</th>
</tr>
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<tbody>
<tr>
<td>3.2</td>
<td>30</td>
</tr>
<tr>
<td>7.8</td>
<td>68</td>
</tr>
<tr>
<td>10.8</td>
<td>100</td>
</tr>
<tr>
<td>14.5</td>
<td>140</td>
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<tr>
<td>22.8</td>
<td>212</td>
</tr>
<tr>
<td>34.0</td>
<td>228</td>
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</table>
DISCUSSION:

The purpose of this investigation was to examine the effects of free fatty acids and the effects of DCA on STZ-diabetic isolated working heart function.

Streptozotocin (60 mg/Kg) was injected into the tail vein of rats in order to destroy pancreatic beta cells, thereby inducing a diabetic state. The diabetic state was characterized by hyperglycemia, hyperlipidemia and a decrease in body weight gain over a six week period (Figures 5-7). Insulin deficiency, due to beta cell loss, results in elevated blood glucose because of a marked decrease in cellular rate of glucose uptake. Elevated blood lipid levels also occur because of an inhibition of lipoprotein lipase and a stimulation of triglyceride lipase, as discussed in the introduction section of this thesis. Diminished body weight gain occurred despite no decrease in food and fluid intake, probably due to dehydration accompanied by protein and fat catabolism (Oakley, 1968).

A time course study on isolated working hearts revealed that cardiac depression occurred between 30 days and 3 months after the onset of diabetes (Penpargkul et al, 1980). Later, it was shown that isolated glucose-perfused hearts from 6-week diabetic rats demonstrated myocardial depression at higher filling pressures, characterized by decreased LVDP and decreased positive and negative dP/dt compared with age-matched controls (Tahiliani et al, 1983). This depression was found
not to worsen with the progression of diabetes (Vadlamudi et al., 1982). Based on the above findings we chose to examine cardiac performance 6 weeks following STZ injection.

We were also able to demonstrate depressed cardiac function in 6-week STZ-diabetic rats compared with age-matched controls. Spontaneously beating diabetic hearts displayed depressed heart rate and HR x PSP when at a medium aortic hydrostatic afterload, otherwise described as a medium workload. Similarly, LVDP was found to be depressed in paced diabetic hearts at a medium workload. Although Ingebretsen et al. (1980) observed only minor differences between the ability of diabetic and normal myocardium to perform work against a low aortic hydrostatic afterload, they demonstrated that increasing the afterload by 70% resulted in the inability of the isolated hearts from diabetic rats to maintain ventricular pressure development. Likewise, we were able to demonstrate a larger depression in LVDP in diabetic hearts compared with controls as the afterload pressure was increased to a maximum (Figures 11, 12). A similar relationship was found when +/-dP/dt were measured in control and diabetic isolated working hearts exposed to increased afterload pressures (Figures 13, 14). These results support the well accepted proposal that a cardiomyopathy exists in the diabetic rat heart and that 6 weeks of STZ-induced diabetes is sufficient time to cause an altered myocardial function.

The pathogenesis of diabetic cardiomyopathy is complex and not well understood. It has been suggested that it may be due, in part, to a combination of changes in myosin and actomyosin ATPase activities (Malhotra et al., 1981), SR Ca$^{2+}$-Mg$^{2+}$-ATPase activity (Lopaschuk et al.,
1983a), sarcolemmal Ca\(^{2+}\) pump activity (Heyliger et al, 1987), and mitochondrial activity (Haugaard and Haugaard, 1964; Pierce and Dhalla, 1987). How these changes occur is uncertain but they have been suggested to be due to metabolic alterations at the level of the heart cell.

As discussed earlier, a major metabolic alteration of diabetes is an increased concentration of circulating fatty acids and concomitant increased reliance of heart cells on fatty acids for oxidative phosphorylation (Denton et al, 1967; Morgan et al, 1961). Some workers believe that this alteration in lipid metabolism may be responsible for some of the biochemical changes that take place in the diabetic myocardium (Tahiliani and McNeill, 1986; Dhalla et al, 1985; Lopaschuk et al, 1983a; Pierce et al, 1983).

Clinically, increased serum FFA levels have been shown to aggravate arrhythmias (Corr et al, 1984) and increase the severity of a myocardial infarction (DeLeiris and Opie, 1978; Liedtke et al, 1978; Vik-Mo and Mjos, 1981). Experimentally, altered lipid metabolism has also been implicated as a contributor to defective heart function. In diabetic rats treatment with lipid-altering drugs such as carnitine (Rodrigues et al, 1988), methylpalmoxirate (Tahiliani and McNeill, 1985), myoinositol (Xiang et al, 1988), and methionine and choline (Heyliger et al, 1986) resulted in an improvement in cardiac function associated with a decrease in various lipid levels. This evidence strongly suggests that lipids may be contributing to diabetic cardiomyopathy.
On an acute basis, it has been shown that i.v. infusion of FFA's into anesthetized intact dogs had no effect on the mechanical activity of the heart (Mjos, 1971), whereas excess FFA's were shown to impair cardiac function in both normal and ischemic isolated working pig and rat hearts (DeLeiris et al., 1978; Henderson et al., 1970; Liedtke et al., 1978; Paulson et al., 1988). We found that the addition of FFA's, in the form of palmitate, to isolated working rat heart perfusate had some detrimental effects on normal and diabetic heart function.

Heart rate and HR X PSP were not affected by the presence of FFA's in the perfusate of spontaneously beating control and diabetic hearts (Figures 8, 9). Similarly, LVDP measured in paced control and diabetic hearts at increasing workloads was unaffected by the presence of FFA's (Figures 10-12). However, in diabetic hearts +dP/dt was found to be depressed by FFA perfusion, at four out of five afterload pressures, but was found to be unchanged in control hearts (Figure 13). Conversely, the presence of FFA's had no effect on -dP/dt of diabetic hearts whereas in control hearts -dP/dt was depressed by FFA's at four out of five afterload pressures (Figure 14). In summary, FFA perfusion had no detrimental effects on control or diabetic isolated working hearts, except for a depressive action on +dP/dt of diabetic hearts and -dP/dt of control hearts.

It is well documented that in the myocardium of the diabetic there is an increased amount of triglycerides (Shipp et al., 1973; Denton and Randle, 1967; Heyliger et al., 1986). This buildup of triglycerides is suggested to occur from increased levels of circulating FFA's resulting in an increased synthesis of, and inhibited hydrolysis of
triacylglycerols (Denton and Randle, 1967; Paulson and Crass, 1982). In addition, total CoA levels of diabetic myocardium are elevated (Reibel et al, 1981), which favours storage of long chain fatty acids as triacylglycerols rather than transport across the mitochondrial membrane and subsequent oxidation (Lopaschuk et al, 1986).

Myocardial triglyceride levels were measured in control and diabetic rats following perfusion with either glucose or glucose and palmitate as available metabolic substrates but no significant difference between any of the groups was found (Figure 25). Knowing that diabetic pre-perfusion triglyceride levels in the heart are high suggests that diabetic hearts are relying on endogenous triglyceride stores for energy production. This proposition is further supported by work done by Wall and Lopaschuk (1989) who demonstrated a decrease in glucose oxidation rates in diabetic hearts perfused in the absence of added FFA's compared with control hearts perfused under the same conditions. They suggest that this occurs due to an increased metabolism of fatty acids from endogenous triglyceride stores. This same group found that the addition of palmitate to the perfusate of diabetic hearts resulted in an almost complete suppression of glucose oxidation. However, a concomitant increase in oxidation of fatty acids was not found. As well, in the presence of palmitate, endogenous fatty acid oxidation from triglyceride stores has not been shown to be elevated in diabetic rat hearts (Paulson and Crass, 1982). Together, this information suggests that diabetic hearts are relying mainly on endogenous stores of triglycerides for energy production with only a small contribution from glucose oxidation.
The finding of a depressive effect on \( +dP/dt \) in diabetic hearts perfused in the presence of FFA's compared with diabetic hearts perfused in the absence of FFA's may be attributed to further depression of glucose oxidation from that which is reported to already exist in the diabetic (Wall and Lopaschuk, 1989). However, the presence of FFA's did not affect HR, HR X PSP, LVDP or \( -dP/dt \) of diabetic hearts, suggesting that energy from endogenous lipid stores was sufficient to maintain most aspects of function at the level of a diabetic heart perfused in the absence of FFA's. But, \( -dP/dt \) was already very depressed in the diabetic perfused in the absence of FFA's, therefore the addition of FFA's would not show a further depressive effect.

Inhibition of myocardial glucose oxidation in the diabetic results from depression of glucose uptake as well as from depression by intermediates of fatty acid metabolism. Citrate accumulation inhibits phosphofructokinase 1, resulting in subsequent depression of glycogenolysis and glucose uptake due to accumulation of glucose-6-phosphate (Newsholme et al., 1962; Kerbey et al., 1985). In addition, an increase in the products/substrates ratio (acetyl CoA/CoA) of PDH, the first irreversible reaction in the mitochondrial oxidative pathway, results in depression of this enzyme and subsequent depression of glucose oxidation (Garland et al., 1962).

The depression of glucose oxidation by fatty acid metabolism has led to much research interest regarding the possibility of increasing glucose oxidation in the diabetic heart. The consequence of this interest has been the development of a number of hypoglycemic drugs which block CPT 1, the enzyme responsible for catalyzing the key
regulatory step in fatty acid oxidation. Recently, drugs in this class, including POCA, the sulfonylurea tolbutamide and Etomoxir, have been demonstrated to increase glucose oxidation rates in isolated working hearts from chronically STZ-diabetic rats (Rosen and Reinauer, 1984; Tan et al., 1984; Wall and Lopaschuk, 1989). In addition to stimulating glucose oxidation in the presence and absence of added palmitate, Etomoxir was shown to significantly improve heart function in palmitate-perfused diabetic rats (Wall and Lopaschuk, 1989).

DCA is another pharmacological agent which has proven capable of increasing myocardial glucose oxidation. The first review of the pharmacologic and therapeutic effects of DCA was published by Stacpoole in 1969. Its reported potential as a hypoglycemic agent made DCA the subject of several investigations in following years.

It is now well established that DCA is an inhibitor of the protein kinase involved in the regulation of the PDH complex, a process which results in PDH activation. DCA was originally demonstrated to activate PDH in rat heart (Whitehouse and Randle, 1973) and was later found to inhibit the ATP-dependent phosphorylation and inactivation of purified pig heart PDH by PDH kinase (Whitehouse and Randle, 1974).

It became apparent that activation of PDH, by DCA, resulted in an increase of glucose oxidation in rat hearts (McAllister et al., 1973; Higgins et al., 1978). Recently, it was shown that direct addition of this agent to isolated working diabetic rat hearts, perfused in the presence of FFA's, stimulates glucose oxidation by 5000% (Lopaschuk and McVeigh, personal communication). Thus, it is apparent that DCA would
be useful in investigating whether a depression of glucose oxidation is contributing to depressed cardiac function in the diabetic.

We investigated the effects of enhanced glucose oxidation on diabetic cardiac function by directly adding 0.5 mM DCA to palmitate-containing isolated working heart perfusate. We were able to demonstrate restoration to control heart values of HR and HR X PSP in spontaneously beating diabetic hearts perfused in the presence of DCA (Figures 15, 16). In paced hearts, DCA was able to restore LVDP and -dP/dt in diabetic hearts to control heart levels at three out of five afterload pressures (Figures 17-19, 21). As well, when perfused in the presence of DCA, +dP/dt values in diabetic hearts were similar to control heart values, at all afterload pressures measured (Figure 20). In summary, results indicate that DCA-induced stimulation of glucose oxidation acutely reversed the depression of diabetic cardiac function.

Why glucose oxidation is important in the heart of the diabetic is not well understood, but a few possibilities have been proposed. A debatable explanation is that glycolytically produced ATP in the cytosol is important for membrane proteins, such as the sarcolemmal ATPases (Bricknell and Opie, 1978). Bunger and co-workers (1986) offer another theory that increased activity of the PDH complex will prevent the accumulation of glycolytic products such as lactate, which may accumulate under ischemic conditions. Another explanation is that less oxygen is required in the production of ATP when glucose is the substrate. It is calculated that complete oxidation of glucose produces 3.17 moles ATP/mole O₂ whereas palmitate produces 2.80 moles ATP/mole O₂ (Lehninger, 1982). It is clear that more work needs to be done to
elucidate the mechanism by which depressed glucose oxidation has a detrimental effect on diabetic heart function.

The reason why DCA acutely reverses the functional changes that occur in the chronically diabetic rat heart is not clear. Previous studies have demonstrated that cardiomyopathic changes in the diabetic rat heart probably occur as a result of alterations in enzymes involved in excitation-contraction coupling, including myosin and actomyosin ATPases activities (Malhotra et al., 1981), SR Ca-ATPase and Ca-transport activity (Lopaschuk et al., 1983a; Penpargkul et al., 1981), sarcolemmal Ca-pump activity (Heyliger et al., 1987) and mitochondrial activity (Haugaard and Haugaard, 1964; Pierce and Dhalla, 1987). If this is the case, then these studies demonstrate that the effects of these changes in enzymatic activity on myocardial function can be masked by acute stimulation of glucose oxidation. These subcellular changes are still probably present but not being manifested as a depression in heart function. The reason why a stimulation of glucose oxidation would overcome the cardiomyopathic changes is not clear. The fact that heart function can be significantly increased in chronically diabetic rat hearts by the acute administration of Etomoxir, however, supports the hypothesis that acute metabolic changes in diabetes are involved in diabetes-induced cardiomyopathies (Wall and Lopaschuk, 1989). To date, very little is known about the relationship between acute changes in myocardial energy substrate utilization and the activities of the enzymes involved in excitation-contraction coupling. It is clear, however, that chronic changes in myocardial metabolism alter the activity of these enzymes. Evidence for this is provided by the
observations that normalization of various aspects of fatty acid metabolism (and the probable normalization of glucose oxidation secondary to these changes) reverse and prevent the changes in heart function associated with diabetes, as well as the activities of many of the enzymes involved in excitation-contraction coupling (Rodrigues et al, 1986; Rodrigues et al, 1988; Tahiliani and McNeill, 1985; Lopaschuk et al, 1983b; Xiang et al, 1988; Heyliger et al, 1986).

Following perfusion, we measured ventricular tissue triglyceride levels in diabetic and control hearts and found a trend towards levels being increased in diabetic hearts perfused in the presence of DCA. However, the increase was not statistically significant (Figure 29). We had reasoned that an increase in triglyceride levels in diabetic hearts following perfusion in the presence of DCA would indicate that the diabetic heart was using glucose as an energy source rather than relying on endogenous stores of triglycerides, as is suggested to occur in the absence of DCA (Wall and Lopaschuk, 1989). However, we were unable to support our hypothesis.

One of the metabolic intermediates of fatty acid metabolism, LCC, has been shown to be elevated in diabetic myocardium (Lopaschuk et al, 1983a) and has been shown to interfere with a number of critical membrane proteins (Lopaschuk et al, 1983; Pitts and Okhuysen, 1984; Shug et al, 1975; Adams et al, 1979). Hence, accumulation of LCC has been implicated as a contributor to depressed cardiac function during diabetes. We measured post-perfusion LCC levels of the myocardium of controls and diabetics perfused in the presence and absence of palmitate and DCA and found no significant changes between any of the groups
(Figures 24, 28). This evidence suggests that this intermediate of fatty acid metabolism is not the major contributor to depressed diabetic heart function.

An important question is whether a decrease in myocardial ATP levels in chronically diabetic rats is contributing to a depression of function. Previous studies have suggested that reduced myocardial function in diabetes is associated with a loss of ATP and adenine nucleotides (Pieper et al, 1984; Rosen et al, 1986). However, in one of these studies, all measured levels of ATP were extremely low (Pieper et al, 1984). In contrast, Lopaschuk and Spafford (in press) found ATP levels were not depressed in hearts from chronically diabetic rats perfused with palmitate. Likewise, we demonstrated that post-perfusion levels of both ATP and creatine phosphate were similar in the myocardium of control and diabetic rats regardless of available substrates or the presence of DCA (Figures 22, 26). This evidence suggests that energy availability is not the limiting factor in diabetic heart function. However, the query as to the relationship between ATP levels and myocardial function in the chronic diabetic requires further investigation.

In summary, we demonstrated that FFA's had no detrimental effects on control or 6-week diabetic isolated working rat hearts, with the exception of a depressive action on +dP/dt of diabetic hearts and -dP/dt of control hearts. We also revealed that addition of the glucose oxidation stimulator, DCA, to palmitate-perfused diabetic isolated working hearts resulted in a marked enhancement of cardiac function. This acute reversal of chronic cardiac depression in the diabetic, by
DCA, suggests that inhibition of glucose oxidation, possibly due to high levels of circulating and endogenous fatty acids, potentiates cardiac function depression in the chronic diabetic.

It is apparent, from this study, that good metabolic control of the diabetic, resulting in normalization of myocardial fatty acid metabolism and, therefore, normalization of glucose metabolism, may be beneficial to diabetic heart function. Chronic control of fatty acid metabolism has been proven to lessen the severity of cardiomyopathy in diabetic animals and, as we have shown, reversal of glucose oxidation depression results in enhancement of cardiac function in diabetic rats. Together, this information suggests that long-term pharmacological therapy aimed at controlling and normalizing fatty acid metabolism is an important clinical goal.
CONCLUSIONS:

1. Six weeks following STZ tail vein injection, rats displayed characteristics of diabetes including hyperglycemia, hyperlipidemia, and decreased body weight gain compared with age-matched control rats.

2. Diabetic animals also exhibited cardiac dysfunction as assessed by a depression in HR, HR X PSP, LVDP, +dP/dt and -dP/dt in the isolated working heart.

3. The presence of 1.2 mM palmitate in the isolated working heart perfusate had no effect on HR, HR X PSP, or LVDP of control or diabetic rat hearts, but had a depressive action on +dP/dt of diabetic hearts and -dP/dt of control hearts.

4. Addition of 0.5 mM DCA to palmitate-containing isolated working heart perfusate acutely reversed diabetic cardiac function depression as assessed by increased HR, HR X PSP, LVDP, +dP/dt and -dP/dt.

5. Post-perfusion ventricular tissue ATP, creatine phosphate, LCC and triglyceride levels were similar regardless of the presence of diabetes or of the presence of palmitate or DCA in the isolated working heart perfusate.
REFERENCES


