THE ROLE OF LIPID ABNORMALITIES IN
THE DEVELOPMENT OF
DIABETIC CARDIOMYOPATHY

By

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We accept this thesis as conforming
to the required standard

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Vancouver, Canada

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ABSTRACT

The incidence of mortality from cardiovascular disease is higher in diabetic patients. Since a significant percentage of the general population is affected by diabetes and a large proportion of the diabetic population suffers from resultant cardiovascular problems, knowledge of the underlying causes and eventual application of the knowledge towards treatment is important. Although a large number of animal models for studying the myocardial problems associated with diabetes are available, the pathogenesis of cardiovascular problems remains relatively obscure. Alterations in sarcolemma, sarcoplasmic reticulum, contractile proteins and mitochondria have been implicated in the development of diabetic cardiomyopathy. However, the metabolic basis of these myocardial effects is unclear. We therefore investigated these metabolic changes in diabetic rat models.

Streptozotocin (STZ) is a broad spectrum antibiotic present in isolates from cultures of *streptomyces achromogenes*. It induces selective pancreatic beta-cell destruction and diabetes in a number of animal species. Diabetes induced by STZ produced hyperglycemia, hypoinsulinemia, hyperlipidemia, polyphagia and polyuria. Six weeks after STZ treatment, hearts from untreated diabetic animals exhibited depressed left ventricular developed pressure (LVDP) and +/- dP/dt compared with control animals. All of these symptoms are similar to insulin dependent diabetes mellitus (IDDM).

However, it could be argued that the cardiac abnormalities observed in animals with STZ-induced diabetes could be due to the direct cardiotoxic effect of the drug or factors not related to the diabetic state. Cardiac sarcoplasmic reticulum (SR) Ca^{2+}-uptake and heart function were hence examined in the BB rat, a strain in which diabetes occurs spontaneously and has been argued to closely resemble insulin-dependent diabetes in humans. Complete insulin withdrawal for 2 or 4 days from BB diabetic rats leads to a spectrum of metabolic derangements including a
loss of body weight, hyperglycemia and elevated triglyceride levels confirming the insulin dependance of this model. BB diabetic rats were treated with a low (hyperglycemic) and high (normoglycemic) insulin dose for 12 weeks after the detection of glycosuria. The hearts from these animals were isolated and SR Ca\(^{2+}\)-uptake and heart function (using isolated perfused working hearts) was examined and compared to BB non-diabetic littermates or Wistar controls. Strain-related differences were found in ATP-dependent SR Ca\(^{2+}\)-uptake between the Wistar and BB rats. There were however no significant differences in SR Ca\(^{2+}\)-uptake related to diabetes between the low dose (LD) insulin treated diabetic group and the high dose (HD) diabetic group or the non-diabetic littermates. Plasma lipid concentrations of the LD and HD BB rats and non-diabetic littermates were also generally higher than those of control Wistar rats indicating strain-related but not diabetes-related differences. In addition, there were no differences in cardiac function between the LD insulin treated animals and the two control groups. These results suggest that overall cardiac contractile function can be maintained with an insulin dose that is not sufficient to correct hyperglycemia. These studies also suggest that since persistent hyperglycemia in the BB diabetic rats treated with low dose insulin (4.5 U.Kg\(^{-1}\).day\(^{-1}\)) for 12 weeks produced no significant physiological abnormalities in the heart, other factors must be contributing to the depression of heart function noted during diabetes. However, it should be pointed out that although this dose of insulin was about 1/3 the amount necessary to optimally control hyperglycemia and glycosuria in BB or STZ-induced diabetic animals, the treated rats showed only moderate signs of insulin lack and therefore experiments with diabetic rats given even less insulin were indicated. Cardiac function was therefore studied in spontaneously diabetic BB rats treated with doses of insulin lower than that used previously. The study involved 2 groups: non-diabetic littermates of BB rats and BB diabetic rats treated daily with a very low insulin dose
(3.5 U.Kg^{-1}.day^{-1}) such that the rats were severely hyperglycemic and hyperlipidemic. The hearts from these two groups were isolated and heart function (using isolated perfused working hearts) and biochemistry were examined, 6 weeks after the onset of diabetes. BB diabetic rats exhibited a lower calcium-stimulated myosin ATPase activity and depressed left ventricular developed pressure, cardiac contractility, and ventricular relaxation rates compared to BB non-diabetic littermates. These results suggest that the chronically diabetic state in the BB rat produces cardiac changes similar to those demonstratable after chemical diabetes induced by alloxan or STZ or those seen during human diabetes mellitus.

Cardiac function was also studied in Wistar-Kyoto (WKY) diabetic rats and diabetic rats treated with hydralazine. WKY diabetic rats did not develop hyperlipidemia or a depressed cardiac function but did show hyperglycemia and hypoinsulinemia. Hydralazine-treatment of Wistar diabetic rats did not alter hyperglycemia or hypoinsulinemia. However, hyperlipidemia and depressed cardiac performance were successfully prevented by hydralazine-treatment. These results, together with those obtained in the BB studies suggest that diabetes-induced hyperlipidemia but not hypoinsulinemia or hyperglycemia may be important in altering cardiac function in experimental diabetic rats. This was studied further using L-carnitine.

L-carnitine is necessary for the transfer of long-chain fatty acids into the mitochondrial matrix where energy production occurs. In the absence of L-carnitine, the accumulation of free fatty acids and related intermediates could produce myocardial subcellular alterations and cardiac dysfunction. Diabetic hearts have a deficiency in the total carnitine pool and develop cardiac dysfunction. This suggested that carnitine therapy may ameliorate alterations in cardiac contractile performance seen during diabetes. The beneficial effects of L-carnitine administration were studied in in vivo and in vitro isolated perfused working hearts.
from control and diabetic rats. Control and STZ-induced diabetic rats were treated daily for 6 weeks with a high (3 g.Kg⁻¹.day⁻¹. i.p.) or low (0.5 g.Kg⁻¹.day⁻¹. i.p.) dose of L-carnitine (prevention study). L-carnitine treatment of the diabetic rats increased myocardial carnitine, significantly reduced plasma glucose and lipid levels and prevented the onset of heart dysfunction in chronically diabetic rats.

Injection of L-carnitine (3 g.Kg⁻¹.day⁻¹. i.p.) for 2 weeks to rats previously diabetic for 6 weeks (reversal study) also partially reversed the adverse effects of chronic diabetes on heart function. In this study, L-carnitine was also a potent lipid-lowering agent. The protective action of L-carnitine on the myocardium appeared to be independent of any direct pharmacological effects. The data suggest that L-carnitine given i.p. can prevent, but at least with the time of treatment used, only partially reverse the myocardial changes seen during diabetes. The mechanism(s) underlying these effects remains to be elucidated but are discussed. Heart function was also studied in STZ-diabetic rats given L-carnitine orally. Oral L-carnitine treatment (50-250 mg.Kg⁻¹.day⁻¹) of 1 and 3 week diabetic rats increased plasma free and total carnitine and decreased plasma acyl carnitine levels. In both groups, myocardial total carnitine levels were increased. However, L-carnitine (200 mg.Kg⁻¹.day⁻¹) treatment of diabetic rats for six weeks had no effect on plasma or myocardial carnitine levels. Similarly, plasma lipids remained elevated whereas cardiac function was still depressed. These studies suggest that in the chronically diabetic rat, the route of administration of L-carnitine is an important factor in determining an effect.

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<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>+dP/dt</td>
<td>Rate of Left Ventricular Pressure Development</td>
</tr>
<tr>
<td>-dP/dt</td>
<td>Rate of Left Ventricular Pressure Decline</td>
</tr>
<tr>
<td>45Ca</td>
<td>Calcium-45</td>
</tr>
<tr>
<td>AMP</td>
<td>adenosine 5'-monophosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine 5'-triphosphate</td>
</tr>
<tr>
<td>BB</td>
<td>Spontaneously Diabetic (Bio-Breeding) Rats</td>
</tr>
<tr>
<td>cAMP</td>
<td>adenosine 3',5'-cyclic monophosphate</td>
</tr>
<tr>
<td>CoA</td>
<td>Coenzyme A</td>
</tr>
<tr>
<td>g</td>
<td>gram(s)</td>
</tr>
<tr>
<td>HD</td>
<td>High Dose Insulin (9-11 U Protamine Zinc Insulin.Kg⁻¹.day⁻¹)</td>
</tr>
<tr>
<td>IDDM</td>
<td>Insulin Dependent Diabetes Mellitus (Type 1)</td>
</tr>
<tr>
<td>LD</td>
<td>Low Dose Insulin (4.5 U Protamine Zinc Insulin.Kg⁻¹.day⁻¹)</td>
</tr>
<tr>
<td>LVDP</td>
<td>Left Ventricular Developed Pressure</td>
</tr>
<tr>
<td>mg</td>
<td>milligram(s)</td>
</tr>
<tr>
<td>min</td>
<td>minute(s)</td>
</tr>
<tr>
<td>PAS</td>
<td>Periodic Acid-Schiff</td>
</tr>
<tr>
<td>SR</td>
<td>Sarcoplasmic reticulum</td>
</tr>
<tr>
<td>STZ</td>
<td>Streptozotocin</td>
</tr>
<tr>
<td>T₃</td>
<td>3,5,3' triiodothyronine</td>
</tr>
<tr>
<td>T₄</td>
<td>3,5,3',5' tetraiodothyronine</td>
</tr>
<tr>
<td>u</td>
<td>micro</td>
</tr>
<tr>
<td>w/v</td>
<td>weight per unit volume</td>
</tr>
<tr>
<td>WKY</td>
<td>Wistar-Kyoto Rats</td>
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</table>
I wish to express my deepest gratitude to my supervisor, Dr. John H. McNeill, whose knowledgeable direction, interest and patience enabled me to complete the work.

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Brian Baltzar Rodrigues
TO MY

MUM and DAD

for their love, caring,
encouragement and support.

Thanks for everything.
INTRODUCTION

1.1. OVERVIEW.

The pancreas is an elongated, pink or yellowish-grey glandular organ composed of an exocrine and an endocrine portion. The exocrine portion of the pancreas comprises about 98% of the organ. The endocrine portion consists of numerous tiny, highly vascular clusters called islets of Langerhans. The islets (about 1 million) are composed of several types of epithelial cells including alpha cells and beta cells. Most of the pancreatic islet cells (60%-80%) are of the beta type and possess many small granules which synthesize and secrete insulin. The insulin molecule is composed of an alpha chain and a beta chain linked together by disulfide bridges. Biologic activity seems to reside in the entire molecular structure rather than specific amino acid groupings within the molecule (Addison-Wesley 1983).

It has long been known that glucose stimulates the beta cells of the pancreas to release insulin which then acts on target cells to promote glucose utilization and storage. Since initially only glucose could be measured, this system was grossly oversimplified; that is, hyperglycemia was believed to be due to insulin deficiency and hypoglycemia was due to insulin excess. With the availability of insulin radioimmunoassays, it became clear that only a minority of patients conform to this scheme; a majority of patients with hyperglycemias are not absolutely insulin-dependent and have a normal or supranormal concentration of circulating insulin. Hence, diabetes mellitus is the name given to a heterogeneous group of disorders with different etiologies. It is characterized by disorders in the metabolism of carbohydrate, protein and fat caused by insulin insufficiency or inadequate insulin action. These abnormalities account for both the acute as well as chronic complications of the disease (for review, see Addison-Wesley 1983).
New research has indicated that there is more than one cause of diabetes and that a great deal of diversity exists in definition, expression and source of the disease. Hence, a new classification was adopted in 1979 and Table 1 summarizes the major categories, current terminology, old labels and major clinical characteristics. As Table 1 indicates, the syndromes which make up diabetes can be broadly divided into two major groups: those associated with insulin-deficiency which account for about 5-10% of all cases (insulin-dependent; IDDM) and those associated with insulin resistance which account for nearly 90-95% of cases (non-insulin dependent; NIDDM). Besides these primary syndromes, there are other types of diabetes which include gestational diabetes (GDM), impaired glucose tolerance (IGT) and diabetes resulting from other conditions or syndromes (National Diabetes Data Group 1979).

1.1.1. Insulin Dependent Diabetes Mellitus (IDDM).

IDDM has been known to man since ancient times and many documents and accounts of experiments are available to explain the origin of this disease (Marble 1971). Closer to our times, the classical series of experiments in pancreatectomized dogs concluded that complete removal of the pancreas caused diabetes (von Mering and Minkowski 1889). It was not a transient glycosuria but a true, lasting diabetes mellitus which, in every aspect, mimicked the worst type of this disease found in man. It was also concluded that diabetes mellitus was associated with an hitherto unknown dysfunction of the pancreas. The possible relationship to the islets of Langerhans did not become evident until several years later. In fact, although pathologists in the early years of this century reported morphological alterations in the islets of Langerhans of patients with diabetes, it was not until much later that Gepts (1965) showed both major abnormalities in the number of beta cells and the presence of inflammatory cells in the islets of Langerhans in newly diagnosed Type 1 (insulin-dependent) diabetic patients. Recent studies with morphometric techniques (Rahier and Goebbels-Henquim 1983;
<table>
<thead>
<tr>
<th>New Category</th>
<th>Old Name</th>
<th>Clinical Characteristics</th>
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<tbody>
<tr>
<td>Type I: Insulin-dependent diabetes mellitus (IDDM),</td>
<td>Juvenile diabetes</td>
<td>- Any age but usually young&lt;br&gt;- Mostly thin at diagnosis&lt;br&gt;- Causes may be genetic or viral but probably involve abnormal immune responses&lt;br&gt;- Often have islet cell antibodies&lt;br&gt;- Little or no endogenous insulin&lt;br&gt;- Need insulin injections to preserve life&lt;br&gt;- Ketosis prone</td>
</tr>
<tr>
<td>5%-10% of all diabetics</td>
<td>Juvenile-onset diabetes</td>
<td></td>
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<tr>
<td></td>
<td>Ketosis-prone diabetes</td>
<td></td>
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<tr>
<td></td>
<td>Brittle diabetes</td>
<td></td>
</tr>
<tr>
<td>Type 2: Non-insulin dependent diabetes (NIDDM),</td>
<td>Adult-onset diabetes</td>
<td>- Any age but usually over 40, occasionally under 21&lt;br&gt;- Cause may be genetic or viral&lt;br&gt;- Mostly obese at diagnosis&lt;br&gt;- No islet cell antibodies&lt;br&gt;- Varying amounts of endogenous insulin, often higher than normal levels present&lt;br&gt;- May need insulin to avoid hyperglycemia&lt;br&gt;- Rare ketosis except in stress or infection</td>
</tr>
<tr>
<td>90%-95% of all diabetics</td>
<td>Maturity-onset diabetes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ketosis-resistant diabetes</td>
<td></td>
</tr>
<tr>
<td>Non-obese - 20% of Type 2</td>
<td>Stable diabetes</td>
<td></td>
</tr>
<tr>
<td>Obese - 80% of Type 2</td>
<td>Maturity-onset diabetes of youth (MODY)</td>
<td></td>
</tr>
<tr>
<td>Impaired glucose tolerance (IGT)</td>
<td>Asymptomatic diabetes</td>
<td>- Blood glucose levels between normal and that of diabetics&lt;br&gt;- Above normal susceptibility to atherosclerotic disease&lt;br&gt;- Renal and retinal complications usually not significant</td>
</tr>
<tr>
<td></td>
<td>Chemical diabetes</td>
<td></td>
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<td></td>
<td>Subclinical diabetes</td>
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<td></td>
<td>Borderline diabetes</td>
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<td></td>
<td>Latent diabetes</td>
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<tr>
<td>Gestational diabetes (GDM)</td>
<td>Gestational diabetes</td>
<td>- Begins or is recognized during or after pregnancy&lt;br&gt;- Above normal risk of perinatal complications&lt;br&gt;- Glucose intolerance transitory but frequently recurs&lt;br&gt;- 50% go on to develop overt diabetes within 15 years&lt;br&gt;- 80% go on to develop overt diabetes after 20 years</td>
</tr>
<tr>
<td>Diabetes mellitus associated with other conditions or</td>
<td>Secondary diabetes</td>
<td>- Accompanied by conditions known or suspected to cause the disease&lt;br&gt;- Certain genetic syndromes</td>
</tr>
<tr>
<td>syndromes</td>
<td></td>
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(Modified from Brunner and Suddarth 1984)
Foulis and Stewart 1984), which allow a quantitative measurement of both the total volume or mass of the endocrine pancreas and of individual cell types, have shown that Type 1 diabetes in man is associated with a specific loss of pancreatic beta cells. The disappearance of beta cells is linked to a diminished mass of the endocrine pancreas, but the total mass of glucagon, somatostatin and pancreatic polypeptide cells seemed to remain within the normal range (Rahier and Goebbel-Henquim 1983). Type 1 diabetes seems, therefore, to represent nature's own beta-cytectomy, a procedure that is possible to carry out using chemical agents like alloxan or streptozotocin.

As Table 1 indicates, the etiology of Type 1 diabetes is not completely understood but probably involves genetic predisposition, viruses and/or an autoimmune response, alone or in combination with each other (Craig 1980). Work done with the human leukocyte antigen (HLA) system that is used in tissue typing has demonstrated a positive relationship with some HLA antigens and diabetes. It has been suggested that the antigens are determinants of the molecular nature of some beta cell surface proteins, some of which may have unique defects that render the cell more susceptible to certain insults (Nerup 1978). The characteristic abrupt appearance of Type 1 diabetes could also be the result of an infection with a diabetogenic virus. A virus similar to, but not identical with, the Coxsackie B4 virus has been identified (Yoon et al. 1979); this virus attacks and significantly damages the pancreatic beta cells. Various studies have also been carried out in animal models and the evidence seems to implicate viruses in the pathogenesis of diabetes (Craighead 1978). Autoimmune factors may also be important in the Type 1 form of the disease. Serum from newly diagnosed Type 1 diabetics contains an antibody capable of complexing with beta cells in the pancreatic islets of non-diabetic, normal individuals. This antibody is found in up to 85% of Type 1 cases at the time of diagnosis (Lernmark et al. 1978).
1.1.2. Non-Insulin Dependent Diabetes Mellitus (NIDDM).

NIDDM patients represent 90-95% of the diabetic population. A large number (between 60 and 90%) of those having NIDDM are obese and they often exhibit hyperinsulinism and associated insulin resistance. Current research strongly suggests that the Type 2 disease originates not as a pancreatic defect but as a result of some receptor defect, either due to a decreased affinity of the receptors for insulin, a "post-receptor defect" or a "down-regulation" of the insulin receptors in response to permanently increased levels of insulin (Kahn et al. 1977; Bar et al. 1978).

1.2. PATHOPHYSIOLOGY OF DIABETES.

Diabetes is known to result in a large number of alterations which affect various systems and organs. These alterations include immediate effects, most of which are reversible with insulin therapy, and long-term effects, which generally appear to take an inexorable course. The short-term effects of insulin deprivation are ketoacidosis, which could progress to lactic acidosis and, rarely, cerebral edema (Young and Bradley 1967) as well as hyperosmolar coma without significant ketoacidosis (Arieff and Caroll 1972) (Figure 1). The long-term pathophysiologic complications of diabetes mellitus include retinopathy and cataract formation, which leads to blindness in patients with long standing diabetes (Davis 1974), nephropathy (Balodimos 1971), neuropathy (Sibley 1982) and cardiovascular diseases.

1.2.1. Heart Disease and Diabetes.

One of the more prominent disorders associated with diabetes is that of heart disease. Cardiac abnormalities occurring as a result of diabetes have been described in various clinical and experimental settings and continue to be the outstanding factor in overall diabetic morbidity and mortality (Goldenberg et al. 1958). A number of clinical studies have shown that the incidence of heart disease is much greater among diabetics
Figure 1

The acute effects of insulin deprivation.
INSULIN DEPRIVATION

- ADIPOSE TISSUE
  - EXCESSIVE RELEASE OF FREE FATTY ACIDS FROM ADIPOSE TISSUE
    - UTILIZED BY SKELETAL AND CARDIAC MUSCLE FOR ENERGY METABOLISM
    - INCREASED LIVER OUTPUT OF GLUCOSE
      - MUSCLE
    - DECREASED ENTRY OF INSULIN INTO INSULIN-DEPENDENT CELLS
      - HYPERGLYCEMIA
        - RENAL THRESHOLD FOR GLUCOSE REABSORPTION EXCEEDED
          - GLYOSOURIA
            - HUNGER
              - WATER LOSS (POLYURIA)
                - SALT LOSS (e.g., NaCl)
                  - LIVER
                    - DEAMINATION
                      - GLUCONEOGENESIS
                        - KETONES
                          - UREA
                            - NEGATIVE NITROGEN BALANCE
      - MUSCLE
        - CATABOLISM EXCEEDS ANABOLISM
          - AMINO ACIDS RELEASED INTO BLOOD
            - LIVER
              - DEAMINATION
                - KETONES

- LIVER
  - FATTY INFILTRATION
    - KETONE FORMATION - ACETOACETIC ACID AND BETAHYDROXYBUTYRIC ACID
      - KETONES IN BLOOD
        - LUNG AND BLADDER
          - ACETONE FORMATION
            - FRUITY ODOUR TO BREATH
              - POLYDIPSIA
                - REDUCED BLOOD VOLUME
                  - SHOCK
        - BRAIN
          - KETOACIDOSIS
            - COMA AND DEATH

- HUNGER
  - WATER LOSS (POLYURIA)
    - SALT LOSS (e.g., NaCl)
      - LIVER
        - DEAMINATION
          - GLUCONEOGENESIS
            - UREA
              - NEGATIVE NITROGEN BALANCE
and now accounts for approximately 80% of all diabetic deaths (Kannel et al. 1974; Kannel and McGee 1979). In fact, diabetics show a two to three-fold increase in the frequency of deaths due to cardiovascular disease when compared to the nondiabetic population.

The pathogenesis of heart failure in diabetes is uncertain. Cardiac disease in the diabetic can result from atherosclerosis but may also be due to a combination of microangiopathy (small blood vessel disease), macroangiopathy (large blood vessel disease), autonomic neuropathy, and other factors which could produce structural, functional, and biochemical alterations in the heart (Ledet et al. 1979). Recently, it has become apparent that cardiac problems associated with diabetes cannot always be definitely implicated with the above factors due to their absence in a significant number of diabetic patients, and studies have now suggested that a specific cardiomyopathy may be a causal factor in producing the increase in mortality and morbidity of diabetes. For example, coronary angiography has made it possible to identify patients with myocardial dysfunction with no significant coronary obstruction. Hamby et al. (1974) found left-ventricular dilatation and hypertrophy in 16 diabetic patients in the absence of systemic hypertension or obstructive large-coronary artery disease. Regan et al. (1977) reported the post-mortem examination of 13 diabetics with no significant coronary disease and showed marked accumulation of Periodic Acid-Schiff (PAS) positive material and triglyceride levels in comparison to nondiabetic normal controls. Regan et al. (1977) also described the hemodynamic findings in four adult diabetics with heart failure and found both left-ventricular systolic and diastolic dysfunction without evidence of ongoing microvascular ischemia. In addition, diabetic men undergoing supine exercise had a lower cardiac output during exercise than did controls, as a result of lower stroke volume (Karlefors 1979). Diabetes was also associated with abnormalities of left-ventricular ejection time and prolongation of the pre-ejection period (Ahmad et al. 1975).
Chemically induced diabetes, using streptozotocin (STZ) or alloxan, also results in myocardial abnormalities which have been studied in animal models. Isolated working hearts from alloxan or STZ-diabetic rats showed a decreased ability to respond to increased left-atrial filling pressures (Miller 1979; Penpargkul et al. 1980; Vadlamudi et al. 1982). In these studies, various indices of cardiac function such as left-ventricular developed pressure, rates of contraction and relaxation, cardiac output, and aortic output were all depressed in response to increased left atrial filling pressures. These results suggest that diabetic hearts have a lower cardiac reserve and, although capable of functioning like controls under normal conditions, may not be able to tolerate situations of high stress and increased venous return. Deterioration of cardiac function was also seen in STZ diabetic rats in the presence of increasing afterloads resulting in a decline of peak left-ventricular pressure and dP/dt (Ingebretsen et al. 1980). Finally, isolated papillary muscles from STZ diabetic rats had a depressed velocity of shortening and delayed onset of relaxation (Fein et al. 1980).

In experimental studies with animals other than rats, Regan et al. (1974), using one-year alloxan-diabetic dogs, showed ventricular stiffening associated with shortening of left-ventricular ejection time. Regan's group also studied chronically diabetic Rhesus monkeys and found similar results; chronic diabetes results in diminished ventricular compliance leading to underfilling (Haider et al. 1981). Fein et al. (1985) examined papillary muscle function in rabbits made diabetic with alloxan and found that the duration of isometric and isotonic contraction and relaxation was markedly prolonged. In most of the above experimental studies, the depressed contractility was not accompanied by decreased myocardial oxygenation, coronary flow or major vessel disease. Since similar observations were made in human studies, it can be proposed that factors besides abnormal blood vessels are involved in the pathogenesis of diabetes-induced heart disease.
The etiology of this functional myocardial abnormality described above is uncertain. Hearts from diabetic dogs appear to accumulate PAS-positive material and collagen which could lead to increased stiffness of the myocardium and thus decreased compliance (Regan et al. 1974). However, increasing evidence suggests that the cardiomyopathy that occurs during diabetes may be due to an alteration of myocardial enzyme systems and subcellular organelles such as sarcolemma, mitochondria, sarcoplasmic reticulum as well as contractile proteins.

1.2.1.1. Sarcolemma.

The cardiac contraction and relaxation cycle is generally viewed as the consequence of raising and lowering the intracellular concentration of free calcium. The importance of the sarcolemma in this beat-by-beat phenomenon is well documented (Dhalla et al. 1976, 1977; Langer 1976). Heart sarcolemma is known to be composed of a basement membrane, also known as the glycocalyx, and plasma membrane. The basement membrane is located on the extracytoplasmic side of the bilayer and contains glycoproteins and mucopolysaccharides (Fawcett and McNutt 1969; Langer 1978). On the other hand, the plasma membrane exists as a bilayer and is composed of phospholipids. Several enzyme and nonenzymatic systems are associated with this lipid bilayer which, together with phospholipids, are involved in the regulation of myocardial ion transport and contractility (Dhalla et al. 1978; Schwartz 1974). Both basement and plasma membranes bind large quantities of calcium which are believed to play a strategic role in the beat-by-beat phenomenon of the heart. When the cardiac cell is depolarized, there is an influx of calcium through the sarcolemma as well as a release of calcium from sarcolemmal stores. The calcium mobilization results in contraction; relaxation, on the other hand, is partly achieved by calcium efflux through the sarcolemma (Dhalla et al. 1978). Some sarcolemmal bound proteins that have been identified as playing important roles either directly or indirectly in myocardial calcium
transport are Na\textsuperscript{+}-K\textsuperscript{+}-ATPase, Ca\textsuperscript{2+}-Mg\textsuperscript{2+}-ATPase (Ca\textsuperscript{2+}-pump), and adenylate cyclase (Dhalla et al. 1978; Schwartz 1974; Caroni and Carafoli 1981). Other calcium transporters such as the Na\textsuperscript{+}-Ca\textsuperscript{2+}-exchanger also appear to have regulatory roles in cardiac contraction (Caroni and Carafoli 1981; Philipson et al. 1982). Thus, from a functional point of view, sarcolemma contains a variety of systems which play important roles in the regulation of myocardial contraction. Hence, any alteration in the composition and structure of this subcellular organelle, either in the basement membrane or plasma membrane, could change its ability to transport calcium effectively and, as a result, the cardiac contraction and relaxation process may be significantly altered.

Several sarcolemmal changes have been identified during diabetes mellitus and are described below.

1.2.1.1.1. Phospholipid and Sialic Acid Changes.

Heart failure associated with diabetes mellitus appears to be accompanied by alterations in sarcolemmal phospholipids (Pierce et al. 1983). In addition to their role as major structural constituents of cell membranes, phospholipids bind large quantities of calcium, which is believed to play a regulatory role in myocardial contraction (Philipson et al. 1980). They also influence several other membrane events, for example, the activity of several enzyme systems. With this being the case, it is conceivable that any alteration in the phospholipid composition of sarcolemma could potentially affect its function. Thus, it is possible that the changes observed in enzyme activities as well as in ion transport systems associated with cardiac sarcolemma during diabetes (Pierce and Dhall 1981; Smith et al. 1984; Makino et al. 1985) might be partially induced by phospholipid alteration.

Phospholipids are also involved in transmembrane methylation. During this process, membrane-bound enzymes convert phosphatidylethanolamine to
phosphatidylcholine by transferring three methyl groups to the amino polar head of the phospholipid (Strittmatter et al. 1981). This methylation of phospholipids alters membrane fluidity, which, according to some reports, influences several enzyme systems. The activities of adenylate cyclase (Hirata and Axelrod 1980) and sarcolemmal-calcium pump (Strittmatter et al. 1979) have been reported to be enhanced during this phenomenon. Phospholipid methylation has been reported to be depressed in the diabetic rat heart and therefore may be involved in the cardiac dysfunction of diabetics (Ganguly et al. 1984).

Defects at the level of the basement membrane (glycocalyx) may also be involved in the cardiac dysfunction seen during diabetes. By virtue of its sialic acid content, the basement membrane is believed to be a site for calcium binding which has been reported to be required for maintaining the structural integrity of the muscle membrane (Langer 1976). In fact, when sialic acid was removed from the sarcolemma, the selective permeability of this subcellular organelle was demonstrated to be disrupted (Langer 1976). It should be pointed out that there is an alteration of sialic acid synthesis in sarcolemma of diabetic hearts (Pierce et al. 1983). This abnormality may be involved in the elevated myocardial calcium levels seen during diabetes (Tansey et al. 1977).

1.2.1.1.2. Enzyme Changes.

\[ \text{Na}^+\text{-K}^+\text{-ATPase} \] This enzyme is believed to be localized in sarcolemma and is considered to control the movement of sodium and potassium across the cell membrane (Schwartz 1974). In fact, according to the current view, this enzyme system maintains the cell membrane in a polarized state. Myocardial contraction is initiated when an action potential depolarizes the sarcolemma. The action potential is triggered by an increase in sodium permeability which results in membrane depolarization. Repolarization is achieved by the outward movement of potassium down its
concentration gradient. It is obvious therefore that a pump system is required to extrude sodium out of the cell and potassium back into the cell in order to maintain the electrochemical gradient necessary to produce a resting membrane potential, which would establish the appropriate environment for subsequent action potentials (Schwartz 1974). Depression of this sodium pump will obviously alter the resting membrane potential and subsequent cardiac contraction. Indeed, Na\(^+\)-K\(^+\)-ATPase activity has been reported to be depressed in diabetic hearts (Pierce and Dhalla 1981). It must be pointed out, however, that inhibition of the Na\(^+\)-K\(^+\)-ATPase system by agents such as cardiac glycosides is believed to be associated with an increase in intracellular calcium concentration (Dhalla et al. 1978). This mechanism seems to implicate Na\(^+\)-K\(^+\)-ATPase inhibition in a cardiac glycoside-induced increase in cardiac contractility. It cannot, however, explain the diminished force of contraction under the pathophysiological situation of diabetes when the enzyme system is also inhibited. Whatever the mechanism by which its inhibition is involved in cardiac dysfunction, an attenuated Na\(^+\)-K\(^+\)-ATPase activity seems to reflect a defect in the failing heart sarcolemmal membrane during diabetes.

**Adenylate Cyclase System:** This system mediates catecholamine and certain other hormonal effects on cells. It consists of at least three distinct components: (1) the receptor which binds the hormone or neuro-transmitter, (2) the catalytic moiety which converts ATP to cyclic AMP (cAMP), and (3) the guanine nucleotide (GTP) regulatory protein which binds and hydrolyzes GTP and which functionally couples the receptor to the catalytic moiety (adenylate cyclase). Upon agonist binding to the receptor, and of GTP to GTP regulatory protein, the catalytic unit is activated and catalyses the conversion of ATP to cAMP. cAMP not only regulates myocardial metabolism but also modulates myocardial contractility by energizing several membrane systems and the contractile apparatus due to phosphorylation induced by protein kinases (Dhalla et al.
1977). Thus, any alteration in this enzyme complex will affect the normal functioning of the myocardium.

Adenylate cyclase activity has been reported to be attenuated in the diabetic heart, and therefore may be partly responsible for the reduced sensitivity of these hearts to catecholamines (Smith et al. 1984; Michel et al. 1985). An alteration in adrenergic receptor density is believed to be involved in this abnormality. A depression in alpha and beta adrenergic receptor number has been reported in diabetic hearts (Heyliger et al. 1982; Latifpour and McNeill 1984). Hence, one of the features of depressed heart function induced by diabetes appears to be a loss of the adrenergic responsiveness of the myocardium.

**Ca\(^{2+}\)-Transporters:** Cardiac contraction is triggered by membrane depolarization and the resulting calcium influx into the sarcoplasm from the extracellular space, as well as calcium release from the sarcoplasmic reticular terminal cisternae. This therefore suggests that excitation-contraction coupling is determined to a large extent by events at the level of the sarcolemma. In fact, the strength of cardiac muscle contraction depends critically on the extracellular calcium concentration (Philipson et al. 1980). The sarcolemma also plays an important role in calcium removal from the sarcoplasm during the relaxation phase of the cardiac contractile process. Sarcolemmal calcium mobilization appears to be achieved by a number of different transporting systems, one being the \( \text{Na}^+\text{-Ca}^{2+}\)-exchanger. It is believed to transport calcium in (Drummond 1979) and out (Reuter 1968) of the cell in exchange for sodium, and appears to be involved in myocardial contraction and relaxation. The \( \text{Ca}^{2+}\)-pump is another sarcolemmal transporter. According to reports, it transports calcium from the sarcoplasm to the extracellular environment and is therefore believed to be involved in cardiac relaxation (Caroni and Carafoli 1981). Both the \( \text{Na}^+\text{-Ca}^{2+}\) exchanger and \( \text{Ca}^{2+}\)-pump of sarcolemma have been reported to be defective in the
diabetic myocardium (Makino et al. 1985) and therefore appear to be involved in the altered calcium transport in the heart during this pathological condition.

1.2.1.2. Mitochondria.

Mitochondria are the chief source of myocardial ATP. This energy, produced by oxidative phosphorylation, is distributed to a number of energy-utilizing systems of the cell, for example Na\(^+\)-K\(^+\)-ATPase and the Ca\(^{2+}\)-pump of sarcolemma and sarcoplasmic reticulum. Although the main function of mitochondria is to generate energy, these organelles have also been reported to actively accumulate calcium under in vitro conditions (Schwartz 1974; Dhalla et al. 1982). Despite their calcium-accumulating ability, it is generally believed that mitochondria do not play a major role in the regulation of cytoplasmic calcium during the contraction-relaxation cycle (Katz 1977). The main arguments against their involvement in effecting relaxation by lowering cytoplasmic calcium concentration are based on their low affinity for that ion, as well as the very slow rate with which they transport physiological levels of intracellular calcium (Katz 1977). Mitochondria have also been shown to exhibit ATP hydrolyzing ability and, even though this ATPase has been isolated and purified from the heart (Bossard et al. 1980), the relationship between mitochondrial ATPase and calcium-uptake activities is far from clear. Studies have also revealed a Na\(^+\)-induced Ca\(^{2+}\) release as well as spontaneous calcium release from mitochondria (Goring et al. 1977). Despite these observations, the physiological significance of this calcium release has not been established. It is believed, however, that the calcium accumulating activity of mitochondria may act as a reservoir or "sink" to modulate intracellular calcium stores (Katz 1977). One drawback to mitochondrial calcium transport is that it competes with its energy-producing activity. This therefore suggests that during pathological conditions, when the myocardium is confronted with abnormal levels of calcium,
mitochondria may not only have to provide ATP for extramitochondrial functions but also transport more calcium via a pathway which competes with ADP phosphorylation.

Calcium transport by mitochondria appears to be disturbed in the heart during diabetes. A depression in calcium uptake activity was observed in the diabetic myocardium, but it returned to control levels after two weeks of insulin therapy (Pierce and Dhall 1985). This impairment in mitochondrial calcium uptake capacity of the diabetic myocardium is similar to that found in various models of cardiac dysfunction and failure.

There are also reports of an alteration in the respiratory activity (which is coupled to ATP production) in mitochondria from diabetic hearts. In the diabetic myocardium, it was observed that phosphorylation of creatine in the presence of succinate or malate was depressed in heart homogenates of alloxan-diabetic rats (Goranson and Erulkar 1949). ATP production was also attenuated in the diabetic myocardium (Haugaard and Haugaard 1964). Similar results were observed by other investigators who found that succinate dehydrogenase activity of the heart was depressed during diabetes (Chen and Ianuzzo 1982). These results seem to suggest that oxidative phosphorylation is reduced in mitochondria of diabetic myocardium.

The consequences of a depression in mitochondrial activity are obvious. A defect in their oxidative respiratory activity may eventually lead to a reduction in ATP levels which, in fact, has been observed in diabetic hearts (Pieper et al. 1984; Pieper and Murray 1987). Low tissue ATP levels may eventually impair normal cellular function and may therefore be involved in the genesis of cardiomyopathy during diabetes.

1.2.1.3. Sarcoplasmic Reticulum (SR).

Calcium transport by the sarcoplasmic reticulum (SR) is a major mechanism by which intramyocardial levels of Ca^{2+} - and thereby, tension development - are
modulated. Participation of SR in such regulatory processes depends on the ability to sequester calcium by an energy consuming process and, as a consequence, promote relaxation of myofibrils (Reuter 1974). Sarcoplasmic reticular membranes contain a $K^+$-sensitive $Ca^{2+}$-dependent ATPase enzyme (MacLennan 1970) which may represent up to 90% of the total protein content of the membrane, and which transports $Ca^{2+}$ from the sarcoplasm into the vesicles of SR with a high velocity and affinity (Chamberlain et al. 1983). Hence, the SR participates in the relaxation of the heart by actively accumulating calcium from the cytoplasmic space, and any alteration in the capacity of this membrane to sequester $Ca^{2+}$ efficiently would, therefore, be expected to have an important impact on the contractile performance of the heart.

In diabetic rat hearts, the ability of the SR to take up calcium is impaired (Penpargkul et al. 1981; Lopaschuk et al. 1983; Ganguly et al. 1983). This may explain the impairment of relaxation in myocardial muscles. Also, as a consequence of lowered uptake, the amount of calcium available for release during the following beats may be lower than normal and thus lead to altered contraction.

Cardiac SR is known to be regulated by a number of factors, among them calmodulin (Katz 1980), cAMP-dependent protein kinase (LaRaia and Morkin 1974), and monovalent cations, particularly $K^+$ (Jones et al. 1977). The $Ca^{2+}$-specific enzyme appears to be regulated either directly via ionic control at allosteric sites or indirectly through phosphorylation of phospholamban, the protein closely associated with the ATPase protein in the membrane. Changes in the concentration of regulatory ions, the degree to which the enzyme recognizes these ions, or the extent to which $Ca^{2+}$-ATPase interacts with phospholamban could all be expected to alter the rate at which the enzyme transports $Ca^{2+}$. Clearly, then, a study of the regulatory changes in the SR $Ca^{2+}$ pump may be important in attempting to elucidate the mechanism whereby a pathological state such as diabetes depresses $Ca^{2+}$-transport activity. However, previous data from our laboratory have suggested that none of the known
regulators of the SR calcium pump plays a role in the depression of cardiac SR Ca\textsuperscript{2+} transport observed in streptozotocin-induced diabetic rat (Lopaschuk et al. 1984). Our laboratory has demonstrated, however, that this calcium-transport defect may result from biochemical changes in the diabetic heart (Lopaschuk et al. 1983).

1.2.1.4. Myosin.

Two cardiac enzyme systems extensively studied during diabetes are myosin and actomyosin ATPases. A number of studies have shown a diabetes-induced depression of the activity these enzymes in rat hearts (Dillmann 1980; Malhotra et al. 1981; Garber et al. 1983; Pierce and Dhalla 1981). This depression was suggested to be due to altered myosin isoenzyme distribution. In non-diabetic control rats, myosin ATPase is present predominantly in the most active V\textsubscript{1} form (about 72% of the total) and about 13% in the V\textsubscript{3} form. The V\textsubscript{3} form is thought to be the slowest of the myosin isoenzymes with regard to ATP hydrolysis as well as cross-bridge formation, and is seen in high concentrations in diabetic rat hearts where the V\textsubscript{1} content is low (Dillmann 1980). Depression of myosin ATPase activity has also been demonstrated in the alloxan-diabetic rabbit (Bhimji et al. 1985). However, in normal rabbits the V\textsubscript{3} form of myosin ATPase predominates, so the mechanism for depression of myosin ATPase would be different from that seen in rats.

Various studies have demonstrated the presence of hypothyroidism in diabetic patients and animals (Dillmann 1980; Pittman et al. 1979). Thyroid hormones, in turn, have a profound effect on the cardiovascular system. While thyroid hormone administration in rabbits and guinea pigs results in increased synthesis of cardiac myosin isoenzyme (Goodkind et al. 1977; Flink et al. 1979), thyroidectomy in rats decreased activity of the enzyme in the heart (Yazaki and Raben 1975). Further evidence suggesting the role of hypothyroidism in diabetes-induced depression of myosin ATPase is provided by studies in which depression of the enzyme in diabetic
rats could be prevented by thyroid replacement therapy (Dillmann 1982). Since activity of myosin ATPase is known to correlate closely with contractility (Scheur and Bhan 1979), it can be hypothesized that diabetes induces a depression of myosin ATPase in the heart which then leads to altered contractility. A summary of the diabetes induced alterations of myocardial enzyme systems and subcellular organelles is depicted in Figure 2.

1.3. MODELS TO STUDY CARDIAC DISEASE DURING DIABETES.

From the above discussion, it becomes apparent that the use of animal models for studies of the cardiovascular complications of diabetes can provide important information. Generally, these animal models of diabetes can be subdivided into two broad categories: those with experimentally induced diabetes and those with spontaneous, genetically determined diabetes.

1.3.1. Drugs and Chemicals that Produce Diabetes.

Insulin secreting cells appear to be more sensitive to chemical insult than other hormone-secreting cells of the pancreas. Knowledge that chemicals can produce diabetes in laboratory animals is long-standing. In the early 1940s, it was found that alloxan, a cyclic urea analog, produced a permanent hyperglycemia in rats (Dunn and McLetchie 1943). This effect was caused by the selective destruction of the insulin secreting beta cells of the endocrine pancreas. The methylnitrosourea analog, streptozotocin, has however replaced alloxan as the primary compound used to produce experimental, insulin-dependent diabetes in laboratory animals (Rakienten et al. 1963). This is due to:

(1) the lower mortality rate seen in streptozotocin-induced diabetic animals (Hoftiezer and Carpenter 1973),

(2) the greater selectivity of streptozotocin for beta cells (Junoud et al. 1969) and
Figure 2

A schematic representation of the events occurring as a result of diabetes, which appear to be the major factors in the development of diabetic cardiomyopathy.
DIABETES MELLITUS

HORMONAL CHANGES

CARDIAC FUNCTIONAL DEPRESSION

A. DECREASED COMPLIANCE OF VENTRICULAR WALL (Deposition of Collagen)

B. MICROVASCULAR ALTERATIONS
   (Small vessel obstruction & diffuse focal necrosis)

C. SUBCELLULAR MALFUNCTION WITHIN THE CELL
   ITSELF.

- CONTRACTILE PROTEIN DYSFUNCTION
  - Shift from myosin isoenzyme V1-V3
  - ↓Ca2+ ATPase activities of myosin and actomyosin

- DEPRESSED SR FUNCTION
  - ↓Ca2+stimulated ATPase activity
  - ↓Ca2+ transport

- DISTURBED MITOCHONDRIAL FUNCTION
  - ↓oxidative capacity
  - ↑Na+,K+ ATPase and Ca2+ uptake

CARDIAC MUSCLE ABNORMALITIES

CARDIOMYOPATHY
(3) the longer half life of streptozotocin in the body (Agarwal 1980).

Although these two agents share the ability to produce beta cell necrosis after single doses in laboratory animals, the mechanisms involved in their effects are not well understood. Streptozotocin, which contains a glucose moiety (2-deoxy D-glucose) coupled to N-methylnitrosourea, is an alkylating agent. This property is certainly capable of initiating its cytotoxic action. It is reasonable to suggest that the glucose moiety present in the molecule directs the alkylating agent to the pancreatic beta cell which may have a special affinity for glucose. In spite of the fact that a unique receptor for glucose in beta cells has not been demonstrated, it has been possible to show that streptozotocin is concentrated in pancreatic islets (Johnson and Tjalve 1978). This concentrating action may account for the cell-selective action of the alkylating agent.

Alloxan on the other hand is taken up by pancreatic cells and rapidly reduced to form dialuric acid. This product undergoes auto-oxidation to yield detectable amounts of \( \text{H}_2\text{O}_2 \), superoxide anion (\( \text{O}_2^- \)) and hydroxyl free-radicals (\( \cdot \text{HO} \)). These reduced species of oxygen, particularly the extremely reactive \( \cdot \text{OH} \) radical, are believed to initiate the alloxan-based attack on beta cells (Malaisse 1982). Alloxan and its metabolites have also been shown to concentrate in pancreatic islet tissue relative to some other tissues. Because alloxan participates in the generation of hydrogen peroxide and the reactive free-radical species of oxygen, it is possible that the alloxan-sensitive beta cell contains unusually low amounts of the protective substances that are present in all cells for the purpose of deactivating reduced forms of oxygen. Of the protective substances that have been measured, only glutathione peroxidase appears to be low in pancreatic islet tissue relative to other tissues (for review see Fischer 1985).
1.3.2. Spontaneously Diabetic BB Rats.

Although there are numerous studies concerning cardiac abnormalities in experimental diabetes induced by STZ or alloxan, there are a few problems with these models. These cell-specific toxins destroy beta cells, provide a relatively permanent diabetes, and are useful because their diabetogenic dose is usually 4-5 times less than their lethal dose. Moreover, the myocardial abnormalities that result from injecting these diabetogenic agents are both prevented (Tahiliani et al. 1983) and reversed (Fein et al. 1981) by insulin therapy indicating that they are due to induced diabetes rather than a direct cardiotoxic effect of these agents. Several limiting factors must however be considered when using the above models of diabetes. For instance, the hyperglycemic action of STZ is markedly influenced by the gender of the animal; males are, in general, significantly more susceptible than females (MacLaren et al. 1980). Susceptibility of animals to STZ is also age dependent, with young rats being more resistant to the diabetogenic action of STZ than older rats (Mordes and Rossini 1981). In addition, alloxan is not entirely specific; lesions occur in other organs and metabolic changes may occur which are unrelated to beta cell necrosis and diabetes (Mordes and Rossini 1981). Alloxan and STZ diabetic rats, although hypoinsulinemic, are also not dependent on exogenous insulin for survival. These animals will survive without insulin and hence do not entirely resemble Type 1 insulin-dependent diabetes mellitus in humans (National Diabetes Data Group 1979).

The spontaneous diabetic syndrome of the BB rat was discovered in 1974 at a commercial breeding facility in a colony of specific pathogen-free, non-obese Wistar rats (Chappel and Chappel 1983). The prevalence of diabetes among the BB rat up to 120 days old varies between 30-50% and susceptible animals become diabetic spontaneously at the age of sexual maturation (60-120 days of age). The development of overt diabetes in this rat (Seemayer et al. 1983), as in man (Gepts 1965), follows massive destruction of pancreatic beta cells and manifests as a diffuse, intense insulitis
associated with a profound reduction of pancreatic immunoreactive insulin. As a model of diabetes, the BB rat is perhaps the closest counterpart to human insulin-dependent (Type 1) diabetes. It develops spontaneously, shows symptoms including weight loss, polyuria, polydipsia, hypoinsulinemia, hyperglycemia, glycosuria and ketoacidosis, and leads to death if exogenous insulin is not administered (Nakhooda et al. 1978; Marliss et al. 1982). These symptoms are similar to those seen during Type 1 diabetes in humans (National Diabetes Data Group 1979). Hence, this genetically defined model of diabetes could have many advantages over other models in which diabetes is induced by injecting drugs or chemicals.

1.4. PREVENTION OF DIABETES-INDUCED MYOCARDIAL ALTERATIONS.

By far, the most successful treatment in preventing and reversing cardiac abnormalities associated with diabetes is that of insulin. The effectiveness of insulin is not totally unexpected since insulin lack is the mainstay of diabetes, and may be the ultimate cause of all secondary complications in the diabetic state. Studies that have investigated the effect of insulin on the performance of cardiac muscles from diabetic rats confirm that the hormone is ineffective in vitro, i.e. when it is included in the organ bath or perfused with the buffer (Tahiliani and McNeill 1986). However, in vivo insulin treatment of diabetic animals was most effective in not only preventing but also in reversing the diabetes-induced myocardial alterations (Tahiliani et al. 1983). Insulin was also effective in preventing or reversing various diabetes-induced biochemical parameters. For example, the change in myosin ATPase activity and the shift in isoenzyme composition was reversed by four weeks of insulin treatment (Dillmann 1980). Studies have also shown that depression in SR calcium transport can be prevented and reversed by insulin treatment (Lopaschuk et al. 1983; Ganguly et al. 1983).
One problem with insulin, however, is that although the myocardial abnormalities associated with experimental diabetes do not seem to worsen as the disease progresses, *in vivo* insulin treatment is not as effective at the more chronic stages (e.g. five months) of diabetes as it is in the less chronic stages (about 6 weeks). Treatment of five month old diabetic animals with insulin for 4 weeks normalized blood glucose levels. However, only partial reversal of cardiac function could be obtained by the treatment (Tahiliani et al. 1983). Similarly, in a chronic canine model of diabetes, collagen accumulation and diminished myocardial compliance were unaffected by insulin control of postprandial hyperglycemia (Regan et al. 1981). Moreover, the myocardial abnormalities seen in various clinical studies exist despite insulin treatment (for review see Dhalla et al. 1985) and tight glucose control. All of these studies raise the obvious and as yet incompletely answered question: Are these cardiovascular changes related to insulin deficiency and hence hyperglycemia, or are factors other than hyperglycemia responsible for the cardiac changes seen during diabetes? Indeed, previous studies from our laboratory have attempted to correlate myocardial alterations in diabetic rats with elevated levels of circulating lipids in the plasma of these animals. The involvement of lipids is suggested by a study in which Wistar and Wistar-Kyoto (WKY) rats were injected with identical doses of STZ. Although both groups of rats had elevated blood glucose levels, only the Wistar animals exhibited elevated levels of circulating lipids. The reasons for this selective protection of WKY rats are not understood. Interestingly, depression of myocardial function was seen in the group of Wistar rats while the cardiac function of the STZ-treated WKY rats remained unaffected (Rodrigues and McNeill 1986). In another study, STZ diabetic rats which were treated with the anti-hypertensive drug hydralazine showed elevated glucose but normal circulating lipid levels. In keeping with the above study, the function of hearts from diabetic rats treated with hydralazine was no different from non-diabetic controls (Rodrigues et al. 1986). Thus it would appear that alterations in lipid metabolism are
an important determinant of cardiac dysfunction during diabetes, the hypothesis being that diabetes causes lipid abnormalities, which in turn lead to an alteration of myocardial enzyme systems and subcellular organelles and this eventually results in cardiac disease.

1.5. LIPID METABOLISM DURING DIABETES.

The purpose of energy metabolism in the heart is to provide an adequate supply of high-energy phosphate compounds to match the continuous use of ATP in contraction and relaxation. Because of the very high turnover rate of ATP in the myocardium, a correspondingly high rate of mitochondrial production of ATP is required. Within the mitochondria, the citrate cycle of Krebs breaks down the critical compound acetyl CoA to CO₂ and hydrogen atoms; the latter in turn yield electrons which are conveyed along the electron transport chain to yield ATP by oxidative phosphorylation before finally combining with oxygen to form water. Acetyl CoA (activated 2-carbon fragment) is derived from one of three major substrates of the myocardium: glucose, lactate and free fatty acids. The uptake of these various substrates by the heart is dependent on the arterial concentration of the fuel concerned. Quantitatively, in normal hearts, cardiac muscle derives a substantial proportion of its ATP from oxidation of fatty acids which generally account for 60 to 70% of oxidative phosphorylation. However, during diabetes, blood lipids have fairly commonly been reported to be elevated and fatty acid utilization by the heart is then altered.

The most commonly elevated fasting plasma lipid level is triglyceride (Nikkila 1974; Rodrigues and McNeill 1986) but plasma cholesterol concentration is also increased during diabetes (Albrink et al. 1963; Rodrigues et al. 1986). Elevation of serum lipids indicates either the defective removal or overproduction (or both) of one or more lipoproteins (Figure 3). Since insulin plays a role in both production and removal of triglyceride-rich lipoproteins, it may be the major cause of lipid disorders of
Lipoprotein pathways and major steps in the regulation of triglyceride metabolism in the adipocyte. Both triglyceride-rich lipoproteins, namely chylomicrons and VLDL, undergo triglyceride hydrolysis by lipoprotein lipase, resulting in the formation of smaller triglyceride-rich lipoproteins. Free fatty acids, released by the action of lipoprotein lipase on triglyceride-rich lipoproteins or from adipose tissue stores, are taken up by many tissues, including adipose tissue, muscle and liver. + Steps where insulin has a positive effect. - Steps which insulin inhibits. LPL, lipoprotein lipase; FFA, free fatty acid; G-6-P, glucose-6-phosphate.

Figure 3
diabetes. There is strong evidence for a defect in the removal of triglyceride-rich lipoprotein in insulin-deficient animals and man that is restored to normal by administration of insulin (Brunzell et al. 1979). Since adipose tissue lipoprotein lipase (LPL) is responsible for the removal of the triglyceride-rich lipoproteins, attention has focused on this enzyme which requires the presence of insulin for maintenance of adequate tissue levels and activity. Indeed, LPL activity has been demonstrated to fall after insulin withdrawal in insulin-dependent diabetic animals and man and is low in poorly controlled diabetic subjects with elevation of serum lipoproteins (Havel 1976).

Another basic biochemical disturbance during insulin deficiency is a marked increase in adipose tissue lipolysis accompanied by enhanced release of free fatty acids (McGarry and Foster 1977). Triglyceride lipase is the rate-determining enzyme in adipose tissue lipolysis (Figure 3). It is an insulin-inhibited, cyclic AMP-dependent enzyme which, under the influence of epinephrine, glucagon and various other lipolytic hormones, hydrolyzes stored triglyceride into diglycerides allowing subsequent release of unesterified fatty acids and free glycerol. The fatty acids thus produced may diffuse out of the cell or they may be re-esterified into triglyceride within the adipocyte. The re-esterification process, however, requires alpha-glycerophosphate which is derived from glucose the influx of which into cells is controlled by insulin. When insulin levels are low, triglyceride lipase acts with much-reduced negative regulation and the result is more rapid net cleavage of triglycerides into free fatty acids and glycerol, both of which are released in large quantities from the adipose tissue into the plasma. Ultimately, with involvement of hepatic lipid synthesis, the net result is hyperlipidemia (Saudek and Eder 1979). Because of this, in diabetic hearts, there is a shift to a predominant fatty acid oxidation and lipids may now account for over 90% of energy production (Denton and Randle 1967). However, because this process takes place in the mitochondria, fatty acids of extra-mitochondrial origin must first be transported from the cytoplasm
through the mitochondrial membrane and then into the mitochondrial matrix. This process occurs via the carnitine-dependent system.

1.6. CARNITINE.
1.6.1. Introduction.

L-carnitine (3-hydroxy-4-N-trimethylammonium butyric acid; Figure 4) was isolated from meat in 1905 (Gulewitsch and Krimberg 1905). While considered neither a vitamin nor an essential nutrient, it is an important cofactor for fatty acid metabolism. This naturally occurring substance has a low molecular weight (MW = 162) and is found in all living tissues and in a variety of food sources (Borum 1983). Studies have shown that both biosynthetic and dietary supply of carnitine are important factors in determining tissue levels of carnitine (Brooks et al. 1985). The dietary sources of carnitine include red meats and dairy products whereas plant-derived foods provide less of this amino acid. When dietary carnitine is low, it can be synthesized endogenously in the body although several essential nutrients (lysine, methionine, ascorbate, niacin, vitamin B₆ and iron) are required. The pathway of carnitine synthesis in mammals is summarized in Figure 5.

Most human tissues make deoxycarnitine but only tissues like the liver, kidney, testes and brain are able to hydroxylate deoxycarnitine to carnitine and export it to the blood in amounts that appear to be adequate for normal metabolic needs (Borum 1983). The hydroxylase catalyzing the last step is missing in the remaining tissues. Virtually all evidence indicates that the enzymatic pathway in rats and humans is identical.

1.6.2. Absorption and Transport.

Absorption of carnitine from dietary sources has not been studied directly. However, Hamilton et al. (1983) studied carnitine movement across human intestinal
Figure 4

Chemical structure of L-carnitine.
Figure 5

Schematic pathway of carnitine biosynthesis: S-adenosylmethionine (SAM) provides the methyl groups for enzymatic trimethylation of peptide-linked lysine. Trimethyllysine (TML) undergoes the transformations as shown.
LYSINE $\xrightarrow{\text{SAM}}$ TRIMETHYLLYSINE (TML) $\xrightarrow{\alpha \text{- KETOGL} + O_2} \xrightarrow{\text{SUCCINATE} + CO_2} \xrightarrow{\text{ASCORBATE, Fe}^{2+}} \xrightarrow{\text{TML}}$ HYDROXY - TML

CARNITINE $\xrightarrow{\text{SUCCINATE} + CO_2} \xrightarrow{\alpha \text{- KETOGL}}$ DEOXYCARNITINE $\xrightarrow{\text{TML}}$ DEOXYCARNITINE ALDEHYDE

SAM = S - ADENOSYL METHIONINE

SCHEMATIC PATHWAY OF CARNITINE BIOSYNTHESIS:

SAM PROVIDES THE METHYL GROUPS FOR ENZYMATIC TRIMETHYLATION OF PEPTIDE - LINKED LYSINE. TML UNDERGOES THE TRANSFORMATIONS AS DETAILED.
mucosa in vitro and concluded that movement of carnitine across this membrane involves an active process that depended on Na\(^+\) co-transport. A passive diffusional process was also identified which may be important for absorption of large doses of carnitine. Carnitine uptake into the isolated perfused adult rat heart also consists of both a saturable (carrier-mediated) and non-saturable (diffusion) component (Vary and Neely 1982). At physiological serum concentrations of free carnitine, approximately 80% of the total myocardial carnitine uptake occurs via the carrier-mediated transport system.

Total carnitine levels in plasma range from 30-90 uM (Rebouche and Engel 1983). At normal physiological concentrations in plasma, more than 90% of filtered carnitine is reabsorbed by the kidneys (Rebouche and Mack 1984) and there are suggestions that under normal conditions, plasma carnitine is regulated, at least in part, by the kinetics of carnitine reabsorption by the kidney.

The concentration of carnitine in tissues is related to the number of mitochondria and capability of oxidizing fatty acids. It is thus paradoxical that tissues richest in carnitine such as heart [which is known to account for the largest consumption of free fatty acids with respect to body weight (Neely and Morgan 1974)] and skeletal muscle are unable to synthesize carnitine and their carnitine content must be met entirely by uptake from the blood for maintenance of intracellular levels.

Inter-organ transport of carnitine precursors and derivatives is also an important process for maintaining tissue levels of carnitine and evidence demonstrating a bidirectional exchange between carnitine and deoxycarnitine across cardiac sarcolemma has been described (Bieber 1989). In this regard, it is assumed that deoxycarnitine is released from muscles into blood, taken up by the liver or kidneys to be hydroxylated to carnitine and the latter returned to muscles (Bieber 1989). It should also be pointed out that each tissue, and perhaps each separate cell type in a tissue, has individual kinetic descriptions for carnitine transport. The liver, for example, has a large capacity
for transport and can double its pool size within a short period as plasma carnitine levels rise. Skeletal and cardiac muscle, although they have high levels of carnitine, do not show rapid exchange of radioactive material or change in concentration in response to changes in plasma carnitine concentration (Gudjonsson et al. 1985).

1.6.3. Excretion.

The portion of a dose of L-carnitine which is excreted in the urine within twenty four hours varies depending on the route of administration. In studies in rats, 95% of a radiolabelled dose of L-carnitine given intravenously or intraperitoneally is retrievable in the urine as unaltered drug. Fecal elimination accounts for less than 2% of the dose (Rebouche et al. 1984). In contrast, after an oral dose, only 34% is recovered as unchanged carnitine with the remainder recovered in the urine as trimethylamine N-oxide (28% dose) and gama-butyrobetaine (31% dose) in the feces (Rebouche et al. 1984). The authors concluded that the extent of metabolism was related to the route of administration and that endogenous flora were responsible for metabolism of L-carnitine taken orally. In humans, a similar situation is seen where the portion of a dose of L-carnitine recoverable in the urine within 24 hours varies depending on the route of administration. After an intravenous infusion, 70% was retrieved in the urine within 12 hours and 80% in 24 hours. In contrast, after an oral dose of L-carnitine, only 7% was recovered in the urine (Welling et al. 1979).

1.6.4. Function.

1.6.4.1. In Fatty Acid Metabolism.

In normal heart muscle, approximately 60% of total energy metabolism comes from the oxidation of fatty acids. Fatty acids are supplied to the heart from the blood where they are carried either as free acid, usually bound to albumin, or as triglycerides in chylomicrons and lipoproteins. The free fatty acid (FFA) is the principal form that is
utilized by the heart. Triglycerides have to be hydrolyzed to FFA by lipoprotein lipase prior to their utilization.

The predominant control of fatty acid uptake in the heart appears to be related to the plasma content of unbound fatty acid which is determined by diet, hormonal control of fatty acid mobilization from liver and adipose tissue and the level of intracellular fatty acid which is determined by the rate of FFA removal by cellular metabolism (Opie 1968).

Fatty acid metabolism requires the presence of both coenzyme A (CoA) and carnitine. CoA is necessary for the cytosolic activation of long chain free fatty acids to acyl CoA esters, which in turn serve as the substrate for beta-oxidation. However, the long chain acyl CoA esters formed in cytosol cannot penetrate the inner mitochondrial membrane for subsequent beta-oxidation (Rebouche and Engel 1983). L-carnitine plays an important role in this process. In the cytoplasm of the cell, fatty acids (acyl groups) are first activated to the CoA esters on the outer surface of the inner mitochondrial membrane in the presence of ATP, Mg\(^{2+}\) and thiokinase. The fatty acyl CoA (FACoA) formed can be used either for synthesis of complex lipids in the cytosol or can undergo beta-oxidation. For beta-oxidation, the acyl groups are transferred from CoA to carnitine with which it combines in the presence of carnitine palmitoyl transferase 1 (CPT1). A specific translocase allows this acyl carnitine to enter the mitochondrial matrix in exchange for either acetyl carnitine or free carnitine. The presence of carnitine palmitoyl transferase 2 (CPT2) on the inner surface of the matrix membrane then reconverts acyl carnitine to acyl CoA. Since CPT catalyses the reversible conversion of long-chain acyl CoA's to long-chain acyl carnitine both in the matrix and cytosolic compartment of mitochondria, two distinct locations for the enzyme must occur.

The acyl CoA within the mitochondrial matrix then enters the fatty acid oxidation spiral (beta-oxidation) and this leads to the production of acetyl CoA which
may enter the Krebs cycle for final oxidation (Goa and Brogden 1987). Short-chain fatty acids can enter the mitochondria by routes not requiring carnitine. A schematic representation of these metabolic events in the myocardium is detailed in Figure 6.

From the above description, it is evident that L-carnitine plays an important role in transporting activated long-chain fatty acids (LCFA) across the inner mitochondrial membrane into the mitochondrial matrix, this being the site of beta-oxidation and Kreb's cycle enzymes. In other words, carnitine acts as a cofactor, allowing acyl groups to be shuttled between intra- and extra-mitochondrial pools of coenzyme A; therefore, adequate levels are required for normal fatty acid and energy metabolism in heart function (Bremer 1983).

1.6.4.2. Other Roles of Carnitine.

Although the function of L-carnitine as an essential endogenous cofactor of fatty acid metabolism is well known, it may also be metabolically important because of its indirect roles. For example, it participates in modulation of the intra-mitochondrial acyl coenzyme A/free coenzyme A ratio (Bieber et al. 1982). This ratio increases as the level of long chain fatty acid increases in the mitochondria. Without a mechanism to limit the delivery of LCFA, the production of LCFA-CoA esters would soon consume the remaining free CoA. The problem with filling up the mitochondria with LCFA-CoA is that it inhibits the function of many other enzymes as will be discussed later. Carnitine also plays an important role in buffering excess and potentially toxic acyl CoA compounds in the body through their conversion to acyl carnitines (Stumpf et al. 1985) which are then eliminated from the kidney (Chalmers et al. 1983).

Increased availability of extracellular fatty acids also raises the rate of fatty acid uptake, but uptake is limited when the capacity to oxidize fatty acids is reached. Under these conditions, the rate of FFA oxidation is limited and acetyl CoA accumulates. Carnitine helps regulate acetyl CoA levels through the enzyme carnitine acetyl
Role of L-carnitine in fatty acid metabolism: scheme to show steps thought to be required to transport extramitochondrial acyl CoA into the mitochondrial matrix.
CIRCULATION

CELL MEMBRANE

THIOKINASE

ATP → FATTY ACRYL CoA → CARNITINE ACYL TRANSFERASE I → (+) FATTY ACRYL CARNITINE → (+) FATTY ACRYL CARNITINE CARRIER

(-) FFA → (+) FFA

CARNITINE → (+) CARNITINE

ACETYL CARNITINE

ACETYL CoA

ATP

INNER MITOCHONDRIAL MEMBRANE

ADENINE NUCLEOTIDE TRANSFERASE

ATP

CITRIC ACID AND OXIDATIVE PHOSPHORYLATION
transferase (which is found in relatively large amounts in cardiac tissue) in that it can provide an alternative pathway for excess acetyl units generated via beta-oxidation. It facilitates formation of acetyl carnitine from acetyl CoA and carnitine (Bieber et al. 1982). The acetyl carnitine formed in the mitochondrial matrix can rapidly exchange with free or acyl carnitine across the inner mitochondrial membrane. By this mechanism, excess acetyl units produced in the mitochondrial matrix may be stored as acetyl carnitine. Under conditions of increased energy demand, the cytosolic acetyl carnitine can be transferred rapidly back into the mitochondrial matrix, providing an additional and readily available source of acetyl units for the citric acid cycle (Figure 6).

The carnitine acetyl transferase reaction may also indirectly regulate the rate of FFA activation to LCFA-CoA by controlling the amount of free CoA available to the cytoplasmic thiokinase reaction. An increase in cytoplasmic acetyl carnitine will increase formation of cytoplasmic acetyl CoA and carnitine by the action of microsomal carnitine acetyl transferase. Consequently, the amount of cytoplasmic free CoA available for thiokinase catalyzed activation of FFA would be decreased. By equilibrating both the mitochondrial and cytoplasmic ratios of acetyl CoA/CoA and acetyl carnitine/carnitine, activation of LCFA is coupled to the rate of beta-oxidation (Figure 6). In addition, by controlling the acetyl CoA/coenzyme A ratio, carnitine may be involved in regulation of glucose utilization, since this ratio is an important regulator of pyruvate dehydrogenase activity (Kerbey et al. 1976).

1.6.5. Lipid Abnormalities During Carnitine Deficiency.

Since the aerobically functioning myocardium derives all its energy from the oxidation of lipids, carbohydrates and ketone bodies (with plasma FFA forming the principal myocardial substrate), cardiac sensitivity to depletion of carnitine is understandable (Bing 1965). A lack of sufficient carnitine to transport LCFA-CoA into the mitochondrial matrix may be the factor responsible for the accumulation of FFA
and related intermediates such as LCFA-CoA and a depression of mitochondrial FFA oxidation (due to the inability of FFA to cross into the mitochondrial matrix and enter Kreb's cycle). These metabolic abnormalities may relate to defects in cardiac performance because high lipid levels in plasma or elevated lipid intermediates in the heart are thought to be potentially noxious to the myocardium. They interfere with various cellular functions by specifically inhibiting enzymes or non-specifically altering structure of membranes by their detergent-like effects (Katz and Messineo 1981). For example, FFA at high concentrations have long been known to increase susceptibility to arrhythmias (Willebrands et al. 1973) and to decrease amplitude of contraction (Liedtke et al. 1978). Similarly, LCFA-CoA inhibits the adenine nucleotide translocator in isolated mitochondria (Vaartjes et al. 1972), a key factor in the regulation of normal ATP homeostasis. This enzyme acts by a "swing-door" mechanism to allow entry of ADP and exit of ATP from the mitochondria. An inhibition of its activity would stop the translocation of ATP from mitochondria to the cytoplasm and ATP availability for contraction of the heart would be impaired. LCFA-CoA are also good detergents and at high concentrations they might be catastrophically disruptive to the heart if left to accumulate (Helenius and Simmons 1975; Corr et al. 1982).

Long chain acyl carnitines (which are known to accumulate during diabetes) also have dramatic effects on the smooth muscle and heart. One example, palmitoyl carnitine has been shown to resemble the Ca\(^{2+}\) channel activator Bay K 8644 in taenia preparations from the guinea-pig caecum (Spedding and Mir 1987). The shift to the left of Ca\(^{2+}\) concentration-response curves (under depolarizing conditions) induced by palmitoyl carnitine was additive with that of Bay K 8644 and pointed to a different molecular site of action in the Ca\(^{2+}\) channel. The interactions of palmitoyl carnitine with different classes of calcium antagonist (it reverses the inhibitory effect of the calcium antagonists) were also similar to those seen with Bay K 8644. The authors concluded that palmitoyl carnitine may interact directly with Ca\(^{2+}\) channels and may
therefore be considered as an endogenous modulator of channel function (Spedding and Mir 1987). Other authors have also shown that palmitoyl carnitine can increase myocardial Ca\(^{2+}\) current, perhaps by modifying Ca\(^{2+}\) channel function in avian ventricular muscle (Inoue and Pappano 1983). This property of the long chain acyl carnitine may then be responsible for the deleterious Ca\(^{2+}\) overload which may occur during diabetes (Dhalla et al. 1985). It must also be pointed out that palmitoyl carnitine has been shown to exert many other direct effects including inhibition of Na\(^{+}\)-K\(^{+}\) ATPase (Adams et al. 1979) and of Ca\(^{2+}\)-ATPase in myocardial sarcoplasmic reticulum (Pitts et al. 1978).

From the above discussion, it becomes apparent that adequate carnitine levels are required for normal FFA and energy metabolism in heart muscle and changes in the levels of carnitine may affect energy production and muscle performance.

1.6.6. Carnitine Deficient States.

Carnitine deficiency can be divided into two broad categories:

1.6.6.1. Primary Carnitine Deficiency.

The primary form of carnitine deficiency was first demonstrated by Engel and Angelini (1973) and can be subdivided into two groups:

**Systemic Carnitine Deficiency:** In this case, the concentrations of carnitine are reduced not only in skeletal muscle, but also in heart, liver and blood. A block in the biosynthesis of endogenous carnitine (as postulated by Karpati et al. 1975), though not excluded, is not considered to be the primary defect by Rebouche and Engel (1984) who suggest additionally,

i) an impairment in the renal or gastrointestinal absorption of carnitine

ii) altered cellular transport (affecting uptake and/or release of carnitine from tissues;
iii) excessive degradation of carnitine.

Myopathic Carnitine Deficiency: This is the best known primary carnitine deficiency (Engel and Angelini 1973) and so far has been recognised in about 30 patients. It is characterized by variable degrees of muscle weakness, coupled with infiltration of lipids within and between skeletal muscle fibres. The carnitine content of muscle is decreased but is normal in liver and blood. This myopathy is thought to arise from a defective transport of carnitine into the muscle (Engel and Angelini 1973).

1.6.6.2. Secondary Carnitine Deficiencies.

Carnitine deficiencies have also been recognised secondary to a variety of genetic defects of intermediary metabolism or other disorders and conditions such as organic acidurias (Chalmers et al. 1984) and chronic hemodialysis (Rebouche and Paulson 1986). A common feature is, or seems to be, an excessive intramitochondrial production of intermediates capable of reacting with CoA, thus creating an abnormally high acyl CoA/CoA ratio. The increase in this ratio may be prevented or modulated by the available free carnitine with formation of acyl carnitine. These acyl carnitines may be excreted from the cell and ultimately appear in the urine (Engel and Rebouche 1984). According to Chalmers et al. (1983), this loss of carnitine may create an increased requirement for free carnitine and thereby a "secondary carnitine deficiency". Whereas primary carnitine deficiencies are characterized by a decrease of both free and total carnitine, secondary carnitine deficiencies might be expected to exhibit a low level of free carnitine and abnormally large amount of esterified carnitine.

The importance of carnitine in cardiac metabolism and function is further emphasized by the growing number of studies demonstrating a close association between systemic and myopathic carnitine deficiency and both hypertrophic and congestive cardiomyopathies, which in some cases can be reversed by carnitine treatment (DiPalma et al. 1979; Tripp and Shug 1984; Whitmer 1987). The observation
that young insulin-treated diabetics have lower total and free serum carnitine (Cedarblad et al. 1982), the evidence that the concentrations of total carnitine (free and acyl) are decreased in the heart and serum of diabetic rats (Vary and Neely 1982), and the correlation between carnitine deficiency and cardiomyopathy suggested that carnitine therapy may ameliorate alterations in cardiac contractile performance seen during diabetes. However, previous studies in our laboratory (Lopaschuk et al. 1983) showed that diabetic rats treated with oral doses of DL-carnitine did not show any improvement in cardiac function, which remained depressed. In any pharmacological study, however, both the dose and the route of administration are important factors in establishing an effect. For example, L-carnitine administered subcutaneously to hypertriglyceridermic Zucker rats at 250-2000 mg.Kg⁻¹.day⁻¹ significantly decreased plasma triglycerides in these obese rats over 8-12 weeks. At the same high dose levels, oral treatment was not effective in decreasing plasma triglycerides (Brady et al. 1986). In addition, our previous study (Lopaschuk et al. 1983) employed DL-carnitine instead of L-carnitine, and studies have now shown that D-carnitine is an antagonist of L-carnitine, the naturally occurring form of carnitine (Leichter et al. 1987).

Paulson et al. (1984) recently showed that daily intraperitoneal administration of L-carnitine for 2 weeks to diabetic rats significantly reduced serum glucose, FFA, triglycerides and ketones, increased total myocardial carnitine stores, and when exposed to varying times of ischemia followed by reperfusion, these hearts were better able to recover contractile performance than saline-treated diabetic rats. In addition, prior in vivo treatment with L-carnitine or providing L-carnitine in the perfusion medium replenished the decreased total myocardial carnitine levels, attenuated the elevation of LCFA CoA and prevented the decline in myocardial ATP levels in acutely diabetic hearts (Pieper et al. 1984; Pieper and Murray 1987). Although the above studies suggested carnitine therapy may be beneficial to the acutely diabetic heart, no studies
have been conducted to test whether long-term treatment with L-carnitine would be successful in treating the cardiac dysfunction seen during chronic diabetes.

1.7. PURPOSE OF THE PRESENT INVESTIGATION.

The overall objective of this thesis was to understand the impact of various biochemical changes on cardiac alterations that occur during chemically induced and spontaneous diabetes mellitus. The following were the major objectives of the investigation:

A) To further study STZ-induced diabetes in the rat as a model of insulin-dependent diabetes.

B) To determine the biochemical and functional alterations in the myocardium of STZ-diabetic rats.

C) Earlier studies utilizing Wistar-Kyoto (WKY) diabetic animals have shown that these rats develop hypoinsulinemia and hyperglycemia but not hyperlipidemia or depressed cardiac function. It was therefore of interest to us to investigate lipid metabolism in these diabetic rats.

D) Earlier studies also suggested that the antihypertensive drug hydralazine, may have a role in regulating lipid metabolism and cardiac performance in diabetic rats. We therefore designed experiments to study the mechanism of action of hydralazine in lowering lipids in these diabetic animals.

E) To study the spontaneously diabetic BB rat as a model of insulin-dependent diabetes. The majority of studies using the BB rat have been directed at characterization of this model i.e. most studies have been carried out on untreated rats until the development of glycosuria. Thus, no studies have been carried out to describe the effects of diabetes on cardiac function in spontaneously diabetic BB rats on long-term insulin treatment. These kinds of studies would be important if the BB rat is to be
used as a model of Type 1 insulin-dependent diabetes. The purpose of this investigation was to study cardiac function and biochemistry in the spontaneously diabetic BB rat treated with different doses of insulin.

F) Since the above and previous studies indicated that hyperlipidemia was a major contributing factor for the development of cardiomyopathy, we studied the effect of L-carnitine (a hypolipidemic agent) on diabetic rats. A variety of doses was used to determine dose-response relationships for carnitine in preventing and reversing the cardiomyopathy. Two routes, oral and intraperitoneal were also studied. In addition, some hearts were perfused with carnitine in order to determine if carnitine could produce a direct inotropic effect.
MATERIALS AND METHODS

2.1. INDUCTION OF STZ DIABETES IN RATS.

Male Wistar rats (200-220 g; Charles River Canada, Montreal, Canada) were transiently anesthetized with diethyl ether to allow injection of either STZ (Sigma, St. Louis, MO) or its vehicle into their tail veins which were dilated by immersion into a beaker of warm water. Diabetes was induced by a single injection of STZ (55 mg/kg) dissolved in citrate buffer (pH 4.5). The buffer was made by mixing 0.1 M citric acid and 0.1 M sodium citrate until a pH of 4.5 was obtained. Control rats were injected with citrate buffer alone. All rats injected with STZ survived and were thereafter housed two to three per cage with Lobound (R) grade corncob bedding (Paxton Processing, Paxton, IL). All rats had access to Purina rat chow and water ad libitum. The lights were left on from 6 AM to 6 PM, keeping in mind that the rats are nocturnal animals. The room temperature was maintained at 25°C.

To obtain a general idea of the severity of the diabetic state, the extent of glycosuria was determined using enzymatic test strips (Tes-Tape, Eli Lilly, Toronto, Canada). To confirm this initial observation, blood samples were taken from the tail vein for blood glucose measurements (Ames: Glucometer and Dextrostix, Ontario, Canada). Three days after STZ injection, those rats displaying glycosuria (greater than 4+) and hyperglycemia (greater than 300 mg%) were used as the diabetic group. The animals were sacrificed 1, 3, and 6 weeks after diabetes induction. At the time of death, whole-blood (arterial and venous) samples were collected from non-fasting animals in heparinized tubes; plasma was separated by centrifugation (3000 X g for 5 min), and then assayed for glucose, insulin and various lipids. Immediately after decapitation, epididymal fat pads were rapidly excised and basal release of glycerol from adipose tissue was determined as described in section 2.1.1. The hearts from control and diabetic animals were removed after six weeks of diabetes and perfused as described in
section 2.6. This duration was chosen based on a report from our laboratory which indicated that alterations in cardiac performance occur six weeks after the onset of diabetes (Tahiliani et al. 1983).

2.2. WISTAR - KYOTO STUDY (WKY).

2.2.1. Animals.

Male Wistar (290-300 g) and WKY (175-180 g) rats (Charles River Canada, Montreal, Canada) were 9 weeks old prior to STZ injections. Diabetes was induced as described in section 2.1. Control rats were injected with citrate buffer alone. We thus had four groups of animals: Wistar controls, Wistar diabetics, WKY controls, and WKY diabetics. The diabetic period was 12 weeks, after which the animals were sacrificed by decapitation. Upon sacrifice, whole blood samples were collected from non-fasting animals in heparinized tubes. The plasma was separated by centrifugation and then analyzed for glucose, insulin and lipid profile. The hearts were removed and perfused as described in section 2.6. Immediately after decapitation, epididymal fat pads were rapidly excised and basal release of glycerol from adipose tissue was determined as described in section 2.11.

2.2.2. Measurement of myocardial triglycerides.

At the end of each perfusion, hearts were frozen between Wollenberger clamps pre-cooled in liquid nitrogen and stored at -70°C until assayed for myocardial triglyceride levels. For this assessment, the frozen hearts were thawed and the atria, fat and aorta were removed. The ventricles were then diced in 25 ml of ice cold homogenization buffer (0.25 M sucrose, 5.0 mM Tris, 1.0 mM MgCl₂, pH 7.4 with HCl) and homogenized with a Polytron PT-10 (3 X 20 sec; setting 5). A 1.0 ml aliquot of this homogenate was used for triglyceride analysis. Lipids were extracted from heart homogenates with a 2:1 chloroform-methanol solution. After aspirating the methanol
and removing the denatured protein from the methanol-chloroform interface, the chloroform in which the lipids were dissolved was evaporated in a stream of nitrogen. The remaining lipid was assayed for triglyceride according to the assay procedure using a Boehringer triglyceride kit. Myocardial protein was quantified with the standard Lowry protein assay (Lowry et al. 1951) using bovine serum albumin as a standard.

2.3. HYDRALAZINE STUDY.

Male Wistar rats (290-300 g; Charles River Canada, Montreal, Canada) were 9 weeks old before STZ treatment. Diabetes was induced as described in section 2.1. Control rats were injected with citrate buffer alone. The rats were then randomly divided into four groups: controls, hydralazine-treated controls, STZ-injected and hydralazine-treated, STZ-injected. Hydralazine was added to the drinking water of the treated animals (0.5-0.6 mg/ml for the controls and 0.08-0.1 mg/ml for the diabetic). Hydralazine intake in mg per Kg per day was determined by the following formula: 1000 X [average fluid intake (ml) X concentration of hydralazine (mg per ml)]/[body weight (g)] (Jadhav and Jandhyala 1983). The animals were sacrificed 6 weeks after the onset of diabetes. Hearts were removed and perfused as described in section 2.6. At the end of each perfusion, hearts were frozen with Wollenberger clamps and stored at -70°C until they were assayed for myocardial triglycerides. Upon sacrifice, whole blood samples were collected from non-fasting animals in heparinized tubes. The plasma was separated by centrifugation and then analyzed for glucose and triglycerides. Immediately after decapitation, epididymal fat pads were rapidly excised and basal release of glycerol from adipose tissue was determined. Lipolysis was also carried out in the presence of varying concentrations of hydralazine and isoproterenol. For these experiments, fragments of adipose tissue from a single animal were pre-incubated for 30 minutes at 37°C. After pre-incubation, these segments were further incubated with fresh medium containing varying concentrations of the test compounds. The incubation
time was one hour at 37°C. Following incubation, the reaction was stopped and the amount of glycerol in the medium was determined as described in section 2.1.1.

2.4. SPONTANEOUSLY DIABETIC BB RATS.

2.4.1. Pilot Study.

An initial pilot study was carried out to determine the effect of complete insulin withdrawal on BB diabetic rats. Spontaneously diabetic male BB rats were obtained from Bio Breeding Laboratories of Canada Ltd. (Animal Resources Division, Health and Welfare, Canada). These diabetic rats were maintained on protamine zinc insulin (9 U.Kg\(^{-1}\).day\(^{-1}\). s.c.) for 2-5 weeks following the development of glycosuria. Non-diabetic litter-mates served as controls. For the experimental groups, no insulin was given for either 2 or 4 days. Body weight was measured daily. Animals were stunned, decapitated, and blood samples were collected for analysis of glucose, insulin and plasma lipids.

2.4.2. BB Diabetic Rats Treated With 4.5 U.Kg\(^{-1}\).day\(^{-1}\) Insulin.

Following the pilot study, SR calcium transport and heart function of thirty male BB rats (approximately 60 days old) were studied. All rats had access to Purina rat chow and water ad libitum. One major difficulty is that this strain of rat is susceptible to the development of pulmonary infection by a variety of organisms found in animal colonies. To prevent the occurrence or spread of infections, it was necessary to keep the rats in barrier housing facilities. The animal room was maintained at 22°C and was supplied with fresh air. There was a photoperiod with a 12-hour light-dark cycle. All animals were maintained in plastic cages on Lobund (R) bedding.

The animals were weighed and monitored daily for urine glucose using enzymatic test strips (Testape; Eli Lilly Co.; Indianapolis, IN). Diabetes was diagnosed on the basis of a positive glycosuria test. Sixty percent of the diabetes prone rats
eventually became diabetic. On detection of glycosuria (designated the day of onset of diabetes), a closer check of the animals revealed weight loss, increase in water intake and urine excretion, and plasma glucose concentrations in excess of 300 mg/dl. In the time period prior to the onset of diabetes, these parameters did not differ from those of litter-mates or non-littermate age matched Wistar controls. On the second day of detection of glycosuria, treatment of the animals was initiated with daily insulin therapy. The injections were given at the same time of the day. To prevent scarring, the injections were given at different locations in the neck. BB diabetic rats were studied on the basis of a low (LD) and high (HD) dose insulin treatment. This was achieved by daily monitoring of urinary glucose and then regulating subcutaneous protamine zinc insulin doses according to daily measurement of body weight. The LD BB diabetic rats received daily injections (s.c) of insulin sufficient to maintain +4 glycosuria (approximately 4.5 U protamine zinc insulin.Kg⁻¹.day⁻¹) using enzymatic test strips. In the case of the HD BB diabetic group, daily injections of 9-11 U of protamine zinc insulin.Kg⁻¹.day⁻¹ were given depending on the glycosuria of individual animals. This prevented hyperglycemia and glycosuria for nearly 24 hours. This daily insulin dose was similar to what has been previously used to optimally control hyperglycemia and glycosuria in BB (Gotfredsen et al. 1985) or STZ-induced (Rasch 1979) diabetic animals. Before injection, the insulin suspension was diluted with distilled water to give the required concentrations. Both groups were administered insulin for a total of 12 weeks after the detection of glycosuria with the last insulin injection occurring 24 hours prior to sacrifice. Age matched Wistar controls and non-diabetic littermates were also sacrificed. During the treatment period, before the injection of insulin, blood samples were taken from the tail vein for blood glucose (Ames: Glucometer and Dextrostix) and plasma insulin (RIA kit) determinations. Upon sacrifice, whole blood samples were collected from non-fasting animals into heparinized tubes. Plasma was separated by centrifugation and then analyzed for glucose, insulin, thyroid hormones (T₃ and T₄)
and lipids. The hearts were removed and used to prepare cardiac SR. In a separate series of experiments, hearts were removed and perfused as described in section 2.6.

2.4.3. BB Diabetic Rats Treated With 3.5 U.Kg⁻¹.day⁻¹ Insulin.

In this study, BB diabetic rats received daily injections (s.c) of insulin (3.5 U protamine zinc insulin.Kg⁻¹.day⁻¹). This daily insulin dose was lower than doses used previously. Insulin was given for a total of 6 weeks after detection of glycosuria. The last insulin injection was given 24 hours prior to sacrifice. At the end of the treatment period, cardiac function was compared with that of non-diabetic littermates. Age-matched Wistar controls and BB non-diabetic littermates were also sacrificed at a similar time point. Upon sacrifice, whole blood (arterial and venous) samples were collected from non-fasting animals in heparinized tubes and then analyzed for glucose, insulin, T₄ and various lipids. Hearts were removed and perfused as described in 2.6.

At the end of each experiment, hearts were frozen with Wollenberger clamps pre-cooled in liquid nitrogen and stored at -70°C, until assayed for myosin Ca²⁺-ATPase.

2.5. CARDIAC SR.

2.5.1. Preparation of Cardiac Microsomes.

Membrane fractions enriched with sarcoplasmic reticulum (microsomal fraction) were made by a modification of the method of McConnaughey et al. (1979). Briefly, atria and connective tissue were removed from hearts and the ventricles were weighed and placed in ice-cold 10 mM sodium bicarbonate buffer (pH 7.4). Ventricles were homogenized in 15 ml of NaHCO₃ (pH 7.4) with a Polytron PT-10 (3 X 15 sec; setting 4). The homogenate was then centrifuged at 500 X g for 5 min. The supernatant was carefully aspirated and following recentrifugation at 7000 X g for 15 min, the supernatant was again centrifuged at 31,000 X g for 30 min. The resulting pellet was
then resuspended in 6-1/2 volumes of 0.6 M KCl and 30 mM histidine chloride (pH 7.0) and centrifuged again at 31,000 x g for 30 min. The final pellet was suspended in 0.25 M sucrose, 0.3 M KCl and 0.1 M Tris (pH 7.2). Previous studies from our laboratory indicated that SR prepared in this way has a low degree of sarcolemmal contamination (Lopaschuk et al. 1983). Sodium azide (5 mM) was added in our assay media to block any mitochondrial ATPase activity. All isolation steps were carried out at 0-5°C, and assay procedures were initiated immediately after isolation. Protein was determined by the method of Lowry et al. (1951).

2.5.2. Measurement of Ca\(^{2+}\) Uptake.

Oxalate-facilitated ATP-dependent Ca\(^{2+}\) uptake into cardiac SR was measured by the method of Tada et al. (1974) with a few modifications. Fresh (unfrozen) microsomal preparations were used for each assay. Oxalate-facilitated Ca\(^{2+}\) uptake was determined in an incubation medium containing 10-30 ug SR protein, 40 mM histidine-HCl, pH 6.8, 5 mM MgCl\(_2\), 5 mM Tris-ATP, 2.5 mM Tris-oxalate, 110 mM KCl, 5 mM NaN\(_3\) and varying free Ca\(^{2+}\) concentrations (range 0.1-2.0 uM). The desired free Ca\(^{2+}\) concentrations were determined by a Fortran program, 'cations', written by Goldstein (1979), and were maintained by addition of varying concentrations of ethylene glycol-bis (beta-aminoethyl ether)-N,N'-tetraacetic acid (EGTA). Samples were pre-incubated for 4.5 min at 30°C, and the reaction initiated by the addition of \(^{45}\)CaCl\(_2\). The reaction was terminated after 5 min by filtering an aliquot of the reaction mixture through a 0.45 uM Millipore filter (HA 45, Millipore). The filter was washed with 15 ml of 40 mM Tris-HCl (pH 7.2), dried, placed in Aquasol (New England Nuclear), and counted for radioactivity in a liquid scintillation counter.
2.6. WORKING HEART PERFUSION.

Animals were stunned with a blow to the head and sacrificed by decapitation. Hearts were quickly excised and placed in cold, aerated (95% O₂:5% CO₂) Chenoweth-Koelle (CK) buffer (pH 7.4), of the following composition (mM): NaCl 120, KCl 5.6, CaCl₂ 2.18, MgCl₂ 2.1, NaHCO₃ 19, glucose 10. After dissecting extraneous tissue free, the aortic stump was located and tied to a 15-gauge stainless steel aortic perfusion cannula. Perfusion with CK buffer (37 ± 1°C) was initiated in a retrograde manner through the aorta at an aortic filling pressure of 45 cm H₂O (30 mm Hg). A 16-gauge stainless steel cannula, connected to atrial filling reservoirs, was then inserted into and tied to the pulmonary vein. Left ventricular developed pressure (LVDP) was measured with a Statham P23 AA transducer (Statham-Gould Instruments) attached to a 3-cm piece of polyethylene (PE-90) tubing. The PE-90 was attached to a 20-gauge needle, which was inserted through the apex of the heart into the left ventricle. Cardiac work was initiated by switching the perfusion system from the retrograde mode to the working heart mode. In the working mode, the perfusate entered the left ventricle through the left atrium and was pumped out through the aortic stump. The aortic outflow was subjected to an afterload of a 75 mm column of H₂O using PE-160 tubing as the column. Left ventricular pressure and the first derivative of left ventricular pressure were recorded on a Grass model 79D polygraph. Each heart was stimulated with a platinum electrode placed on the left atrium at twice the threshold voltage with square-wave pulses of 5 msec duration (from a Grass model SD9D stimulator) to give a rate of 275 beats/min. Cardiac function data [left ventricular developed pressure (LVDP), rate of force development (+dP/dt) and rate of relaxation (-dP/dt)] were collected and analyzed with a microcomputer (Apple II with a Mountain hardware board). The pressure transducer signal from the polygraph was sampled at 667 Hz over 1.5 sec at each function curve. This resulted in data being collected for six complete cardiac pulses. Three of these were analyzed with curve-
fitting techniques to determine pulse height, area, start and finish. The values from these three pulses were averaged to produce the data at that point. The hearts were equilibrated at 15 cm H$_2$O atrial filling pressure for 10 minutes before the function curves were performed by estimating the left ventricular function against varying left atrial filling pressures. Different filling pressures were obtained by changing the height of the left atrial filling reservoir from 5.0 to 22.5 cm in 2.5-cm steps. The filling pressure was first reduced stepwise from 15.0 to 5.0 cm H$_2$O, after which it was increased stepwise to 22.5 cm H$_2$O and finally decreased stepwise to 15.0 cm H$_2$O. At each point, pressure development was allowed to stabilize before it was recorded. In general, stable pressure development was achieved within two minutes after left atrial filling pressure was changed. A complete function curve was usually performed in about 20-30 minutes. The total time of perfusion of each heart was approximately 45 minutes. At the end of each experiment, hearts were frozen between Wollenberger clamps pre-cooled in liquid nitrogen and stored at -70°C until assayed.

2.7. CONTRACTILE PROTEIN.

Myosin was purified from rat hearts and Ca$^{2+}$-activated ATPase activity of myosin was determined by a previously described method (Bhan and Malhotra 1976). Briefly, hearts were pulverized and homogenized in 0.05 M KCl and 0.01 M KPO$_4$ (Buffer 1, pH 6.8) and then centrifuged at 3000 X g for 10 min at 4°C. The pellets, consisting of myofibrils were further treated with 0.05 M KCl and 0.002 M EGTA (pH 7.0) followed by centrifugation (3000 X g, 10 min, 4°C). The pellets obtained were homogenized in Buffer 1 containing 1% Triton X-100 (twice). Myosin was then extracted and isolated from the myofibrils with 10 volumes of high-salt pyrophosphate [0.47 M KCl, 0.01 M KPO$_4$ and 0.02 M Na pyrophosphate (pH 6.8)] for 20 mins. This was followed by fractionation with a saturated (NH$_4$)$_2$SO$_4$ solution containing 5 mM MgCl$_2$ and 10 mM EDTA. The fraction precipitating between 35 and 45% (NH$_4$)$_2$SO$_4$
was collected, dissolved in high salt (0.3 M KCl) and dialyzed overnight against 0.3 M KCl and 1 mM EDTA (pH 7.0) in order to eliminate (NH₄)₂SO₄. All steps were carried out in the presence of 0.1 mM PMSF (phenyl methyl sulfonyl fluoride). Hearts of diabetic animals were always extracted and analyzed simultaneously with hearts of controls on which the same reagents and incubation conditions were used. Myosin obtained in this manner was shown by SDS gel electrophoresis to be free of actin, troponin, and tropomyosin and to be without evidence of proteolytic degradation (Malhotra et al. 1981).

The Ca²⁺-activated ATPase of myosin was determined in a final volume of 1.0 ml at 30°C. For the Ca²⁺-dependent ATPase activity of myosin, the incubation buffer contained 0.507 M KCl, 0.05 M Tris (hydroxymethyl)-aminomethane (pH 7.6), 0.01 M CaCl₂, 0.005 M DTT (dithiothreitol), 0.005 M Na₂-ATP and 0.1 mg/ml of myosin. 0.05 M EGTA was added to the above reaction mixture to determine the basal ATPase activity in the absence of Ca²⁺. All ATPase activity measurements were initiated by the addition of Na₂-ATP and terminated after 10 min by the addition of 0.5 ml cold 10% trichloroacetic acid (TCA). Inorganic phosphate was determined by the method of Fiske and Subbarow (1925). Protein concentration was determined by the Biuret method using bovine serum albumin as a standard (Layne 1957). Results are expressed as umoles phosphate per mg of protein per minute.

2.8. BLOOD ANALYSES.

Plasma collected was stored at -20°C until assayed. Plasma glucose, phospholipids, triglycerides, and total cholesterol were determined using Boehringer Mannheim diagnostic reagent kits. Plasma immunoreactive insulin, T₃ and T₄ were assayed using radioimmunoassay kits obtained from Amersham.
2.9. MEASUREMENT OF OUABAIN-SENSITIVE Na\textsuperscript{+}-K\textsuperscript{+}-ATPase ACTIVITY.

Ouabain-sensitive Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity was assayed in a medium containing 50 mM Tris-HCl (pH 7.4), 100 mM NaCl, 10 mM KCl, 4 mM MgCl\textsubscript{2}, 1 mM EDTA, 5 mM NaN\textsubscript{3}, and 4 mM ATP at 37°C for 10 min. The activity was calculated as the difference between ATP hydrolysis measured as inorganic phosphate released with and without 2 mM ouabain present (Ganguly et al. 1983).

2.10. L-CARNITINE STUDY.

2.10.1. Prevention Study.

Male Wistar rats (200-220 g, Charles River Canada, Montreal, Canada) were used in this study. Diabetes was induced as described in section 2.1. The rats were then randomly divided into four groups: saline-treated control, L-carnitine-treated control, saline-treated STZ-injected, and L-carnitine-treated, STZ-injected. The L-carnitine salt (3g.Kg\textsuperscript{-1}.day\textsuperscript{-1}) dissolved in saline was injected intraperitoneally (i.p) starting 3 days after STZ administration. The injection sites were varied because the rats tended to develop superficial lesions due to the osmotic properties of L-carnitine, which were more evident in the L-carnitine-treated control rats. At sacrifice, no gross evidence of trauma was evident within the abdominal cavity as a result of repeated injections. The injections were given at 5 PM everyday. A high dose of carnitine (L-carnitine inner salt, Sigma) had to be administered because carnitine distributes from the plasma to the liver, kidney and spleen at a faster rate than to cardiac muscle (Brooks and McIntosh 1975). In addition, a similar dose administered to diabetic rats has been shown to decrease glucose, FFA and triglyceride levels and to increase total myocardial carnitine content (Paulson et al. 1984). Control animals received an equal volume of 0.9% NaCl solution. The rats were treated for 6 weeks with the last injection given 24 hours before sacrifice. Hearts were removed and perfused as described in section 2.6. At the end of each perfusion, hearts were frozen between Wollenberger clamps pre-cooled in liquid
nitrogen, and stored at -70°C until assayed for free carnitine levels. After sacrifice, whole blood samples were collected from non-fasting animals in heparinized tubes, plasma was separated and then assayed for glucose, insulin, T₃, T₄ and various lipids.

2.10.2. Reversal Study.

The aim of this study was to investigate whether L-carnitine treatment was capable of *reversing* the abnormalities after they had occurred. This was in contrast to our earlier study (section 2.10.1.) where we investigated whether L-carnitine treatment, initiated three days after STZ injection and continued for six weeks, was capable of *preventing* the cardiac changes from occurring. The rats were divided into four groups: saline-treated controls, L-carnitine treated controls, saline-treated STZ injected and L-carnitine-treated, STZ-injected. L-carnitine salt (3 g.Kg⁻¹.day⁻¹) dissolved in saline was injected i.p. starting six weeks after STZ administration. The rats were treated for two weeks with the last injection given 24 hours before death. In effect, animals were sacrificed eight weeks after the induction of diabetes. Hearts were removed and perfused as described in section 2.6. Immediately after decapitation, epididymal fat pads were quickly excised and basal release of glycerol from adipose tissue subsequently determined. Blood was collected and analyzed as described in section 2.8.

2.10.3. Low Dose L-Carnitine Study.

For this study, the rats were divided into three groups: saline-treated controls, saline-treated STZ injected and L-carnitine-treated, STZ injected. The L-carnitine salt (0.5 g.Kg⁻¹.day⁻¹), dissolved in saline, was injected i.p. for six weeks starting three days after STZ administration. The last injection was given 24 h before death. This dose was selected on the basis of earlier studies which indicated that rats treated chronically with very high doses of carnitine (2-3 g.Kg⁻¹.day⁻¹. i.p.) tend to develop superficial lesions due to the osmotic properties of L-carnitine (Brady et al. 1986; Rodrigues et al.
1988) and to lose weight (Rodrigues et al. 1988). Keeping this in view, and the observations that rats receiving L-carnitine develop hepatic lesions, it cannot be assumed that a high dose of carnitine is totally innocuous (Brady et al. 1986). In addition, recent studies have shown that prior in vivo treatment with as little as 0.2 g.Kg⁻¹.day⁻¹ L-carnitine attenuates the elevation of long-chain fatty acyl CoA and prevents the decline in myocardial ATP levels in acutely diabetic hearts (Pieper et al. 1984; Pieper and Murray 1987). Hearts were removed and then perfused as described in section 2.6. At the end of each experiment, hearts were frozen with Wollenberger clamps pre-cooled in liquid nitrogen and stored at -70°C until assayed for myosin Ca²⁺-ATPase. Blood was collected and analyzed as described in section 2.8.

2.10.4. Direct Effects of L-Carnitine on Cardiac Function.

This study was undertaken to determine the dose-related effects of carnitine perfusion on cardiac function. Rats were divided into two groups: control and STZ-injected. After six weeks, the animals were sacrificed by decapitation. Hearts were excised quickly and cannulated for perfusion in the working-heart mode. Briefly, the hearts were equilibrated at 15 cm H₂O left atrial filling pressure for 10 min with Chenoweth-Koelle (CK) buffer. After equilibration, the perfusion medium was switched to CK buffer containing varying concentrations of L-carnitine up to 1 mM. Any given heart was exposed to only one concentration of L-carnitine for a total duration of twenty minutes. In another series of experiments, control and diabetic hearts were equilibrated at 15 cm H₂O left atrial filling pressure for 10 min with CK buffer. After equilibration, the perfusion medium was switched to CK buffer containing varying concentrations of L-carnitine up to 1 mM and function curves were performed as described in section 2.6. During the function curve, any given heart was exposed to only one concentration of L-carnitine.
2.10.5. Effects of Oral L-Carnitine Treatment.

**Pilot Study:** An initial pilot study was carried out to determine an effective dose of L-carnitine. Diabetes was induced in male Wistar rats (200 - 220 g, Charles River Canada, Montreal, Canada) as described in section 2.1. The rats were randomly assigned into two groups: controls and STZ injected. The diabetic rats were subdivided into different groups which were given varying doses of L-carnitine (0, 50, 150 and 250 mg.Kg\(^{-1}\).day\(^{-1}\)). L-carnitine was added to the drinking water of the treated animals. L-carnitine intake (mg.Kg\(^{-1}\).day\(^{-1}\)) was calculated using the formula of Jadhav and Jandhyala (1983).

One and three weeks after L-carnitine treatment was begun, the animals were sacrificed. At the time of sacrifice, whole blood samples were collected, plasma was separated by centrifugation and then assayed for glucose, insulin, various lipids, T\(_4\) and carnitine.

**STZ Diabetic Rats Treated with 200 mg.Kg\(^{-1}\).day\(^{-1}\) L-carnitine given Orally:**

Our pilot study established that oral doses of L-carnitine (between 50 - 250 mg.Kg\(^{-1}\).day\(^{-1}\)) were effective in elevating total and free carnitine levels in diabetic rats, one and three weeks after diabetes induction. Following the pilot study, we then selected a high dose (200 mg.Kg\(^{-1}\).day\(^{-1}\)) and conducted experiments to assess the effect of this dose of L-carnitine given orally on cardiac function in chronically (6 weeks) diabetic rats. The animals were divided into four groups: controls, L-carnitine-treated controls, STZ-injected and L-carnitine-treated, STZ-injected. The intake of L-carnitine for both control and diabetic groups was calculated daily and the concentration adjusted to get the dose. Since diabetic rats consumed a large volume of fluid, controls were given a higher concentration of L-carnitine to produce the same dose of L-carnitine as in diabetic rats. All rats were treated for six weeks and then sacrificed. Hearts were removed and perfused as described. Blood samples were also
collected at the time of sacrifice and assayed for glucose, insulin, T₄, carnitine and various lipids.

2.11. PREPARATION OF FAT PADS AND LIPOLYSIS.

Both epididymal fat pads were obtained after the animals were sacrificed, and washed in warm Krebs-Ringer bicarbonate buffer (KRB) of the following composition (mM): NaCl 125, KCl 5, CaCl₂ 1, MgCl₂ 2.5, KH₂PO₄ 1, Tris HCl 25 and glucose 2 mg/ml. Segments (0.5-0.8 g) of fat bodies were cut freehand with scissors, pre-incubated in KRB buffer (pH 7.4) for 30 min and finally incubated in 5 ml KRB at 37°C in a metabolic shaker (60 cycles/min) under an atmosphere of 95% O₂-5% CO₂ for 1 hr. Each incubation was run in duplicate. At the end of incubation, the reaction was stopped with perchloric acid (final concentration 3%) added to the incubation medium. The incubation medium was neutralized with KHCO₃, centrifuged and the supernatant frozen at -20°C until analyzed for glycerol. Basal glycerol release (umoles.g⁻¹ of tissue.hr⁻¹) was used as an index of lipolysis and was measured enzymatically using a Boehringer Mannheim glycerol kit.

2.12. CARNITINE ANALYSIS.

2.12.1. In Plasma.

Samples were assayed for free and total carnitine [using the radioisotopic procedure of Parvin and Pande (1977), later modified by Seccombe et al. (1978)] using carnitine palmitoyl transferase.

**Free carnitine:** Free carnitine in plasma was determined directly. The plasma (100 ul) was placed in an ice bath. ZnSO₄ (0.087 M) and Ba(OH)₂ (0.083 M) were added for deproteinization. Equal volumes of the above two solutions were used and this resulted in a supernatant of pH 7.0. The sample was centrifuged (3,000 rpm, 15 min, 4°C) and the supernatant was used for determination of free carnitine.
**Total carnitine:** The total carnitine levels were assayed as free carnitine after alkaline hydrolysis of an aliquot of plasma (100 ul). In this case the sequence of addition of ZnSO$_4$ and Ba(OH)$_2$ was reversed. Following the addition of Ba(OH)$_2$, the tubes were stoppered and transferred to a 75°C water bath for 45 mins (resultant pH greater than 12.0). Subsequently, the tubes were cooled on ice and equal volumes of ZnSO$_4$ were added. The precipitate was removed by centrifugation (3,000 rpm, 15 min, 4°C) and an aliquot of the supernatant was used for the determination of total carnitine.

**Acyl carnitine:** The amount of acyl carnitine was determined by subtracting free carnitine from total carnitine levels.

2.12.2. In Heart.

Frozen heart tissue was pulverized 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.

**Free carnitine:** Extraction of free carnitine was done directly according to the method of Idell-Wenger et al. (1978). Approximately 50 mg of frozen powder was extracted into 2 ml of ice-cold perchloric acid (6% w/v) containing 15 mM DTT. This tissue-perchloric acid mixture was centrifuged (2,000 rpm) at 4°C, and a portion (0.5 ml) of the acid soluble supernatant was neutralized with 6 N KOH (without prior hydrolysis) and used for the determination of tissue levels of free carnitine fraction according to the method of Parvin and Pande (1977).

**Total carnitine:** Total L-carnitine in the heart was assayed as free carnitine after alkaline hydrolysis of the powdered tissue using the method of Paulson et al. (1984). Approximately 50 mg of powdered frozen heart tissue was sonicated in 1 ml 0.5 M KOH (pH 12.5 to 13.0) until dissolved. This mixture was subjected to alkaline hydrolysis by incubation for 60 min at 50°C. After hydrolysis, the solution was cooled
on ice for 5 min. The pH of the solution was adjusted to 7.0 - 7.4 with 0.25 M MOPS-HCl, the solution centrifuged (2,000 rpm, 5 min, 4°C) and the supernatant analyzed for free carnitine using the method of Parvin and Pande (1977). This value includes carnitine present originally as free carnitine as well as the free carnitine resulting from the hydrolysis of all esterified forms or total myocardial carnitine.

2.12.3. Assay Procedure.

**Principle:** The following reaction forms the basis of the assay:

\[
\text{Carnitine} + \text{Acetyl-CoA} \rightarrow \text{Acetyl-carnitine} + \text{CoA}
\]

\[
\text{Carnitine-acetyltransferase}
\]

The reaction was stopped after 1 hour using charcoal (which removes the unreacted excess Acetyl-CoA). To prevent the reverse reaction, N-ethylmaleimide (NEM) was added, which binds to CoA formed during the reaction. Acyl carnitine undergoes hydrolysis at alkaline pH, therefore the supernatant must be near pH 6.8 - 7; otherwise, falsely elevated levels of free carnitine will be attained.

**Assay procedure:** To 100 ul of prepared sample, 150 ul of enzyme/assay mixture (containing NEM, [acetyl-\(^3\)H] acetyl CoA, unlabeled acetyl CoA, HEPES/EDTA and carnitine acetyl transferase) was added. All samples were assayed in triplicate. The tubes were incubated for one hour in a water bath (25°C). The reaction was stopped with a charcoal slurry, tubes were placed on ice and then centrifuged (3,000 rpm, 15 min, 4°C). The centrifugation was repeated using the supernatant. To 500 ul of the final supernatant, scintillation fluid was added and counted for radioactivity in a liquid scintillation counter (Seccombe et al. 1978).

Since the hearts were perfused, the values for free carnitine are expressed on a dry-weight basis. In addition, the perfusion medium did not contain carnitine and therefore the values obtained represent actual tissue values.
2.13. STATISTICAL ANALYSIS.

Results are presented as mean ± S.E.M. (standard error of mean). The statistical differences between mean values for the various groups were evaluated by two way analysis of variance. Newman-Keul's test was applied to determine differences between means within the population. For Ca$^{2+}$-uptake and heart function curves, repeated measures of analysis of variance was used. All other analyses were carried out using paired or unpaired Students' t-tests where appropriate. A probability of $P = 0.05$ was taken as the level of statistical significance.
RESULTS

3.1. GENERAL FEATURES OF STZ-DIABETIC RATS.

Rats treated with STZ exhibited symptoms characteristic of the diabetic state. Diabetic rats did not gain as much weight as age-matched controls and had significantly lower body weights at the time of sacrifice (Figure 7A). The difference between the diabetic and control rat body weights appeared to be due to a loss of fat depots, emaciation of skeletal muscle, and a smaller skeleton. This occurred despite the fact that diabetic animals had dramatically elevated fluid (polydipsia) and food (hyperphagia) intake (Figure 7, A and B). An observed distension of their stomachs with food, at the time of sacrifice, also suggested the typical hyperphagia of insulin-dependent diabetes. Diabetic rats also had increased fecal and urine output which were not measured quantitatively.

Urine glucose was qualitatively assessed three days after STZ injection using Tes-Tape. On a scale of 1+ (0.1%) to 4+ (2.0%), it was found to be between 3+ and 4+ for most of the rats. No detectable glucose was present in the urine of control animals. Plasma glucose levels of diabetic animals were found to be elevated (3-4 fold) as early as one week after diabetes induction and remained elevated for the remainder of the study (Figure 8). On the other hand, plasma insulin levels measured at the time of death were more than 50% depressed in diabetic animals at all time points (Figure 8).

Figure 9 shows plasma lipid profiles in the experimental rats. Plasma triglyceride levels were elevated in diabetic rats at every time point measured (Figure 9A). Plasma cholesterol levels were also increased (Figure 9C), but the increase was not as marked as in the case of triglycerides. Basal rates of glycerol output from diabetic adipose tissue were more than doubled in about three weeks following diabetes induction and remained elevated.
Cardiac performance at various left atrial filling pressures was also studied in isolated perfused working hearts. Rats diabetic for six weeks developed significantly lower LVDP when compared with age-matched control rats (Figure 10). Similar results were also obtained for the left ventricular +dP/dt and -dP/dt.

3.2. WISTAR-KYOTO STUDY.

Injecting Wistar or WKY rats with STZ resulted in a diabetic state, with an elevated urine glucose (>2%) throughout the study period. No detectable glucose was present in the urine of control animals. Parameters used to assess the diabetic state of animals are summarized in Table 2. At the time of sacrifice, plasma glucose levels were markedly elevated in STZ-injected rats accompanied by a depression in plasma insulin levels. Plasma free fatty acids, triglycerides, total cholesterol and phospholipids were also significantly elevated in the Wistar diabetic group. In contrast, plasma lipid levels of the WKY diabetic rats appeared to be normal after 12 weeks (Figure 11).

In Wistar rats, basal rates of glycerol output were increased from diabetic adipose tissue as compared to controls. However, in both diabetic WKY and corresponding control rats, basal glycerol release from adipose tissue was similar (Figure 12). Figure 13 indicates myocardial triglyceride levels, 12 weeks after STZ injection, in Wistar and WKY control and diabetic rats. While the triglyceride levels in the myocardium of Wistar diabetic rats were greater than in controls, there was no difference in these levels for control and diabetic WKY rats.

To investigate the possibility of a relationship between lipid abnormalities and contractile characteristics, positive and negative dP/dt of hearts from control and diabetic rats were measured (Figure 14). At a filling pressure of 15.0 cm H$_2$O, the hearts from Wistar diabetic rats showed lower +/- dP/dt compared with Wistar controls. WKY diabetic rats, however, did not show such a depression in cardiac
3.3. HYDRAZONE STUDY.

Hydralazine treatment of STZ (described in section 3.1) rats had no effect on the high glucose levels in their plasma (Table 3). In contrast, the elevated plasma triglyceride levels were markedly reduced by this treatment (Table 3). This lipid lowering effect of hydralazine did not appear to be due to an effect on adipose tissue lipolysis. The elevated basal glycerol release from diabetic adipose tissue, remained unaffected by hydralazine (Figure 15).

To confirm that the lipid lowering effect of hydralazine was not due to a direct effect on adipose tissue lipolysis, adipose tissue fragments were incubated with increasing concentrations of hydralazine. This treatment did not change the basal rate of lipolysis in either control or diabetic rats (Figure 16). Figure 16 also shows the effect of isoproterenol on the lipolytic response of adipose tissue from normal and STZ-diabetic rats. Isoproterenol increased glycerol release in both control and diabetic rats. The in vitro effect of hydralazine on isoproterenol-stimulated lipolysis was evaluated next. As Figure 16 indicates, hydralazine, even at very high doses, did not alter the lipolytic response of isoproterenol.

As Figure 17 indicates, the myocardial triglyceride levels in hearts, 6 weeks after STZ injection, were increased in diabetic rats. In hydralazine-treated diabetic rats, the triglyceride levels were higher than control values but lower than in diabetics.

Figure 18 shows the effect of varying filling pressures on LVDP. Hearts from untreated diabetic rats showed lower LVDPs when compared with untreated controls at all filling pressures. Hydralazine treatment of the diabetic rats restored the LVDPs to normal, but only at filling pressures greater than 12.5 cm H2O. These results confirmed the observation that untreated diabetic rats are characterized by a depressed cardiac
performance. Hydralazine treatment of diabetic rats brought about a definite improvement in their depressed cardiac performance and a concomitant decrease in their plasma and myocardial lipid levels.

3.4. SPONTANEOUSLY DIABETIC BB RATS.

3.4.1. Pilot Study.

In the pilot study, urine glucose showed 4+ sugar values (Tes-Tape) for BB diabetic animals within 24 hours of withdrawal of insulin. The impact of insulin withdrawal even for 2 days was significant enough to cause marked metabolic alterations. For instance, the diabetic rats showed a rapid and progressive decrease in body weight accompanied by dehydration despite hyperphagia and polydipsia (Figure 19, Table 4). Hyperglycemia developed within 2 days (Table 4) and was responsible for the glycosuria. It was interesting to note that the insulin dose given to prevent glycosuria was associated with hyperinsulinemia (Table 4). Alternatively, on insulin withdrawal for 2 or 4 days, immunoreactive insulin was still measurable (Table 4). The effect of insulin withdrawal on fat metabolism expressed itself as a significant rise in plasma triglycerides (Figure 20). However, there was no significant increase in total plasma cholesterol or phospholipids (Figure 20). In addition, ketosis occurred along with a marked wasting of body tissue, including fat and muscle protein. This suggested that body lipid stores were depleted, an observation confirmed by a visible loss of adipose tissue at sacrifice. Thus, most of the features of diabetes in the spontaneously diabetic BB rats were similar to those seen after STZ injection in rats. This is not surprising if the apparent relative selectivity of the spontaneous syndrome for the beta-cells is taken into consideration.
3.4.2. BB Diabetic Rats Treated With 4.5 U.Kg\(^{-1}\).day\(^{-1}\) Insulin.

*General Features of the Experimental Rats:* For the cardiac studies, most of the diabetic animals had an onset of clinical symptoms at an average age of 100 ± 3 days with a range of 77-124 days. No detectable glucose was present in the urine of BB non-diabetic littermates or age-matched Wistar controls. Of the 30 rats used, 18 became diabetic. After detection of glycosuria in the affected animals, the disease was characterized by rapid weight loss, polyuria and polydipsia. The response to a high dose (HD) of insulin was dramatic with a marked reduction in urine glucose output, normal blood glucose levels (Table 5, Figure 21), and the resumption of normal weight gain and growth throughout the study period (Table 5). The low dose (LD) rats, on the other hand, had persistent hyperglycemia and hypoinsulinemia throughout the study period (Figure 21, Table 5).

*Criteria Used to Assess the Diabetic State of Experimental Rats:* One major difference between the STZ model of diabetes and the spontaneous syndrome of diabetes treated with LD insulin was the degree of hyperlipidemia. In the former, triglyceride and cholesterol levels were markedly elevated (Rodrigues and McNeill 1986). However, in the BB rat given LD insulin treatment sufficient to maintain glycosuria (2-4+), the same magnitude of hyperlipidemia did not occur (Figure 22). Thus, although plasma total phospholipids, cholesterol and triglyceride concentrations in the BB rat groups were generally higher than in age-matched Wistar rats, there were no significant differences between the BB rat groups themselves. In addition, although the LD insulin rats developed hyperglycemia, they did not show a reduction in T\(_3\) or T\(_4\) levels when compared with either control (Table 5). This was in agreement with other reports (Sternathal et al. 1981; Wright et al. 1983) but was in contrast to the STZ model of diabetes in Wistar rats which showed reduction in the levels of these hormones when compared with non-diabetic Wistar controls (Rodrigues and McNeill 1986).
Cardiac Alterations in the BB Diabetic Rats: ATP-dependent, Tris-oxalate-facilitated SR $\text{Ca}^{2+}$-uptake was examined over varying free $\text{Ca}^{2+}$ levels in the various experimental groups (Figure 23). When comparing Wistar control rats with BB non-diabetic littermates, repeated-measures analysis of variance showed that there was a significant group-by-treatment interaction, indicating that the effect of $\text{Ca}^{2+}$ on SR $\text{Ca}^{2+}$-uptake was dependent on group membership (i.e. Wistar control or BB non-diabetic littermate) ($P<0.05$). Subsequent repeated measures analysis of variance of the three BB rat groups showed that there were no significant group-by-treatment interactions. That is, the effect of the diabetic state on SR $\text{Ca}^{2+}$-uptake was not dependent on group membership. The results indicated strain-related, but not diabetes-related, differences in $\text{Ca}^{2+}$-uptake activity in the BB rat. The yields of SR (mg/gm wet ventricle) obtained from the various groups were similar: Wistar Control $2.40 \pm 0.08$; BB non-diabetic littermates $2.39 \pm 0.15$; HD BB diabetic $2.30 \pm 0.11$; LD BB diabetic $1.93 \pm 0.18$. To determine whether or not the observed alterations in SR function in the BB rat groups were a consequence of a differential purity of these fractions, ouabain sensitive $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity of purified SR vesicles was measured. The activity was found to be minimal in the different groups. It must also be pointed out that $\text{Ca}^{2+}$ uptake was measured in the presence of 5 mM NaN$_3$, an inhibitor of mitochondrial ATPase activity (Ganguly et al. 1983). Thus, the difference in the $\text{Ca}^{2+}$-uptake activity in SR from BB as compared to Wistar rats was not influenced by the presence of varying amounts of subcellular contaminants in these preparations.

Figures 24A-24C indicate the effect of varying filling pressures on the parameters of heart function (LVDP, $+\frac{dP}{dt}$ and $-\frac{dP}{dt}$) in Wistar control, BB non-diabetic littermate and low-dose BB diabetic rat groups. Repeated measures analysis of variance of the 3 groups showed that there was no group-by-treatment interaction. That
is, the effect of the diabetic state on cardiac function was not dependent on group membership.

3.4.3. BB Diabetic Rats Treated With 3.5 U.Kg$^{-1}.day^{-1}$ Insulin.

Diabetes in the poorly controlled BB diabetic animals was characterized by severe hyperglycemia (>300 mg/dl) and elevated urine glucose (>2%) throughout the study period. No detectable glucose was present in the urine of BB non-diabetic littersmates or age matched Wistar controls. As before, body weights of diabetic animals were lower than those of control animals (Table 6) despite hyperphagia and polydipsia.

Various parameters used to assess the diabetic state of animals are summarized in Table 6. Insulin levels were measured in plasma obtained at the time of death. Interestingly, BB non-diabetic littermates had lower insulin values compared to Wistar rats and yet their blood glucose values were similar. In contrast, BB diabetic rats treated with insulin had measurable amounts of immunoreactive insulin but had persistent hyperglycemia throughout the study. BB diabetic rats had similar plasma T$_4$ levels to those in BB non-diabetic littermates. However, myosin Ca$^{2+}$-ATPase activity in the BB diabetic rat hearts was lower than the littermate controls.

The effects of low insulin on fat metabolism are presented in Figure 25. BB diabetic rats treated with 3.5 U.Kg$^{-1}.day^{-1}$ insulin showed markedly elevated levels of triglyceride. However, although plasma cholesterol in the two BB rat groups were higher than in age-matched Wistar rats, there were no significant differences between the BB rat groups themselves.

When perfused at various atrial filling pressures, the LVDP in isolated working hearts from BB diabetic rats was significantly depressed at filling pressures higher than 12.5 cm H$_2$O (Figure 26C). Similar effects were noted with cardiac contractility. BB diabetic rats showed lower +dP/dt values than BB non-diabetic littermates at filling pressures greater than 17.5 cm H$_2$O (Figure 26A). The differences in ventricular
relaxation rates between the two groups were not as apparent. Although -dP/dt values in the BB diabetic hearts were lower as compared to BB non-diabetic littermates, the differences were not statistically significant except at the highest filling pressure (Figure 26B).

3.5. L-CARNITINE STUDY.

3.5.1. Prevention Study.

*General Features of the Experimental Rats:* Injecting animals with STZ resulted in a diabetic state characterized by hyperglycemia (>300 mg/dl) and an elevated urine glucose greater than 2% throughout the study period when the animals were left untreated. These symptoms were evident as early as 24 hours after STZ injection. No detectable glucose was present in the urine of control animals. Surprisingly, when the animals were treated with L-carnitine and the extent of glycosuria was measured weekly using enzymatic test strips, seven of the eight STZ-diabetic rats showed no detectable glucose in their urine. Weight gain during the study period was significantly less in both diabetic groups than in control animals, such that diabetic rats showed significantly lower body weights at the time of death. L-carnitine-treated control animals also gained weight more slowly than untreated control rats, such that there was a significant difference between the groups after 6 weeks (Figure 27). Although food intake by these animals was not measured, the weight loss in the L-carnitine treated control animals was a concern. Untreated diabetic rats drank more water than controls rats, but this polydipsia was prevented by L-carnitine treatment (Table 7). Table 7 also shows that both diabetic groups exhibited reduced heart weights compared to untreated control animals.

*Criteria Used to Assess the Diabetic State of the Experimental Rats:* Various criteria used to assess the diabetic state of the animals are summarized in Table 8. Plasma insulin levels measured at the time of death were depressed in the untreated
diabetic rats and L-carnitine treatment of diabetic rats did not increase the insulin levels (Table 8). However, L-carnitine treatment significantly decreased the elevated plasma glucose values in the diabetic rats (Figure 28). This reduction was presumably due to a mechanism independent of insulin, which remained depressed in these animals. Plasma T4 levels were significantly lower in the untreated diabetic rats. The L-carnitine treated diabetic rats did not show such a reduction when compared with either control group. Similarly, the T3 levels were reduced in the untreated diabetic animals, whereas the L-carnitine-treated diabetic rats showed a definite improvement in the levels of this hormone. Diabetic hearts had lower free carnitine levels when compared with controls. Prolonged high dose L-carnitine treatment of diabetic rats restored the myocardial free carnitine levels to normal. Thus, it was evident that STZ treatment resulted in a diabetic state and that the use of L-carnitine was an effective method of improving the depressed thyroid status in diabetic rats and reducing the elevated glucose levels.

*Effect of L-Carnitine on Control and Diabetic Rat Hearts:* Figures 29 - 31 show the effect of varying filling pressures on various indices of heart function (LVDP, +dP/dt and -dP/dt) in diabetic and control rats. Hearts from untreated diabetic rats showed lower LVDP, +dP/dt and -dP/dt than hearts from untreated control rats at left atrial filling pressures greater than 10 cm of H2O. L-carnitine treatment of the diabetic rats restored the LVDP (Figure 29) and had similar effects on +dP/dt (Figure 30) and -dP/dt (Figure 31). The concentration of L-carnitine used in this study had no deleterious effect on the cardiac performance of control rats. In fact, although not significantly different, both L-carnitine-treated control and diabetic rats showed levels of cardiac function higher than that of control rats. These results confirmed the fact that untreated diabetic rats are characterized by depressed cardiac performance. In contrast, the performance of the hearts from L-carnitine-treated diabetic rats did not differ from that of control rats.
**Change in Plasma Lipids After L-Carnitine Treatment:** Plasma triglycerides, total cholesterol and phospholipids were markedly elevated in the untreated diabetic group (Figure 32). L-carnitine treatment of the diabetic rats restored these parameters to normal. The plasma lipids of L-carnitine treated controls were normal. Thus, it appeared that L-carnitine controlled the elevated plasma lipids of diabetic rats, and had no effect in the control rat.

3.5.2. Reversal Study.

Despite hyperphagia and polydipsia, diabetic rats showed a rapid and continuous decrease in body weight gain (Figure 33). After L-carnitine administration the control rats began losing weight such that after 2 weeks of treatment, their body weights were significantly lower than those of untreated control rats (Figure 33). This weight loss in the L-carnitine treated control animals was a concern. When food intake was measured in various groups, the results indicated that diabetic rats generally ate more than controls (Figure 34B). However, on L-carnitine treatment, food intake immediately dropped in both control and diabetic animals. This could explain the observed weight loss. Untreated diabetic rats drank more water than controls but this elevated fluid intake was reduced by L-carnitine treatment (Figure 34A).

Various indices used to assess the diabetic state of the animals are summarized in Table 9. L-carnitine treatment of diabetic rats for two weeks significantly decreased the elevated plasma glucose values in untreated diabetic rats. Treatment with L-carnitine also restored the markedly elevated plasma triglyceride, total cholesterol and phospholipid levels in the untreated diabetic group to normal. Basal glycerol output rates were more than doubled from diabetic adipose tissue in comparison to control rats (Table 9). L-carnitine administration for two weeks decreased the basal glycerol output of diabetic rats, with no effect on controls.
Figure 35 shows the effect of varying filling pressures on heart function (LVDP) of control and diabetic rats. Hearts from untreated diabetic rats showed lower LVDP compared with untreated controls at left atrial filling pressures greater than 12.5 cm H₂O. L-carnitine treatment of diabetic rats for 2 weeks improved but did not completely reverse the depressed heart function seen in untreated diabetic rats.

3.5.3. Low Dose L-Carnitine Study.

Both diabetic groups showed significantly lower body weights at the time of sacrifice (Table 10). Food intake measured in various groups indicated that diabetic rats ate more than controls (Table 10). However, even with the low dose carnitine treatment, diabetic rats ate less than untreated diabetics. Untreated diabetic rats drank more water than controls and this increased fluid intake was lowered by L-carnitine treatment (Table 10).

Various measurements used to assess the diabetic state of the animals are summarized in Table 11. Low dose L-carnitine treatment for 6 weeks significantly decreased plasma glucose, triglycerides and phospholipids which were elevated in the untreated diabetic group. This was also seen in our previous study (section 3.5.1). However, low dose carnitine was ineffective in lowering the elevated plasma cholesterol of diabetic rats. T₃ levels were reduced in the untreated diabetic animals. L-carnitine treatment of diabetics showed a definite improvement in the levels of this hormone. Table 11 also indicates that myosin Ca²⁺-ATPase remained depressed in L-carnitine treated diabetic rats.

Hearts from untreated diabetic rats showed lower LVDP and +dP/dt compared to untreated controls at left atrial filling pressures greater than 17.5 cm of H₂O (Figure 36A & 36B). Low-dose L-carnitine treatment of diabetic rats completely restored the LVDP to control values (Figure 36A). Similar effects of L-carnitine treatment were noted on +dP/dt (Figure 36B). These results confirmed the fact that untreated 6 week
diabetic rats have depressed cardiac performance. In contrast, the performance of hearts from L-carnitine-treated diabetic rats did not differ from controls.

3.5.4. Direct Effects of L-Carnitine on Cardiac Function.

Cardiac function of perfused hearts from both normal and diabetic rats is shown in Figure 37, A and B. Although different concentrations of L-carnitine were used, only the highest concentration perfused is indicated. For both group of hearts, the presence of L-carnitine even at a very high dose (1 mM) induced no significant changes in heart function. The improvement in cardiac function by L-carnitine could thus not be explained on the basis of its direct action on the heart.

3.5.5. Oral L-Carnitine study.

Our initial experiments involved treating animals with oral doses of L-carnitine from 50-650 mg.Kg\(^{-1}\).day\(^{-1}\) for one week. However, increasing the dose beyond 350 mg.Kg\(^{-1}\).day\(^{-1}\) had no beneficial effects on body weight gain (Figure 38) or plasma lipids. Hence, for the sake of brevity, only the results with 50, 150 and 250 mg.Kg\(^{-1}\).day\(^{-1}\) L-carnitine are discussed.

_Pilot study:_ Plasma carnitine levels (free, acyl and total) of one week diabetic rats are shown in Figure 39. Total plasma carnitine content of control and diabetic rats were similar. In contrast, circulating free carnitine decreased by approximately 35% whereas the percentage of acyl carnitine increased almost two fold, when compared with control rats. In other words, the equilibrium between free and esterified carnitine is shifted towards increased esterification. These changes are reflected by the acyl-carnitine/carnitine ratio which is raised in the plasma of diabetic rats (Table 12). Table 12 also shows total carnitine content of hearts from control and diabetic rats. Diabetic hearts had approximately 30% less carnitine per gram dry tissue when compared with controls one week after diabetes induction. The above results were also observed with
three week control and diabetic rats (Figure 40, Table 13). However, in this study, diabetic hearts had approximately 46% less carnitine per gram dry tissue when compared with controls three weeks after diabetes induction.

The effects of treatment of the animals with oral L-carnitine are given in Figure 39 and 40. L-carnitine supplementation for one week resulted in a clear change in the carnitine status. The plasma fractions of total and free carnitine increased for the 150 and 250 mg.Kg\(^{-1}\).day\(^{-1}\) dose groups; however, acyl carnitine remained elevated (Figure 39). For the three week treatment period, all doses studied showed elevation of total and free carnitine. At this time period the relative percentage of the esterified carnitine decreased. It thus appeared that the total carnitine pool in the L-carnitine-treated animals was the result of a significant increase in the levels of free carnitine and not acyl carnitine (Figure 40). In both studies, L-carnitine treatment (especially the two higher doses) prevented a decrease in myocardial total carnitine content (Tables 12 and 13).

The response of plasma triglycerides and cholesterol to L-carnitine administered orally is shown in Tables 12 and 13. The conclusions from both one and three week studies was that L-carnitine administered orally to diabetic rats decreased plasma triglycerides with no effect on plasma cholesterol. Tables 12 and 13 also show the effect of L-carnitine on blood glucose and \(T_4\) in control and diabetic animals. The significant elevation in blood glucose in diabetic animals in both studies, were not prevented by L-carnitine treatment. However, after a minimum of 3 weeks, L-carnitine treated diabetic rats showed an elevation of plasma \(T_4\).

The conclusions from these pilot studies were that acute oral L-carnitine administration to diabetic rats improves their carnitine status and reduces plasma levels of triglycerides. Whether chronic oral L-carnitine therapy would be of benefit to diabetic rats was investigated next. 200 mg.Kg\(^{-1}\).day\(^{-1}\) of L-carnitine was selected as the dose for this study.
**Chronic oral treatment with** 200 mg.Kg$^{-1}$.day$^{-1}$ **L-carnitine:** The general features and indices used to assess the diabetic state of experimental rats are given in Table 14. Injecting animals with STZ resulted in a diabetic state as described previously. Oral L-carnitine treatment of these diabetic rats had no effect on body weight gain or fluid and food intake (which remained depressed). Similarly, in these treated rats, plasma lipids and glucose remained elevated whereas insulin and T4 values were still lower than in control groups.

Plasma carnitine levels (free, acyl and total) of six week diabetic rats are shown in Figure 41. Unlike the one and three week studies, plasma total carnitine levels of diabetic rats were now reduced as compared to controls. In these diabetic rats, circulating free carnitine decreased (by approximately 48%) whereas the percentage of acyl carnitine increased almost two-fold. The effects of treatment of the diabetic animals is also given in Figure 41. Oral L-carnitine supplementation for six weeks had no effect on the carnitine status; total and free carnitine levels remained depressed whereas acyl carnitine remained elevated.

Figure 42, A to C shows the effect of varying filling pressures or parameters of heart function (LVDP, +dP/dt and -dP/dt) in the four groups. Repeated measures analysis of variance showed that L-carnitine treatment of diabetic rats had no effect on cardiac performance which remained depressed.

### 3.6. FIGURES AND TABLES

The figures and tables are presented in the following pages.
Figure 7

Time course of body weight increases in control and diabetic rats (Panel A, line graph). Food (Panel A, bar graph) and fluid intake (Panel B) of the two groups are also shown. Values are expressed as mean ± SEM ($n = 8-10$ animals). Stars represent significant differences from the control group ($P<0.05$) [Section 3.1].
Figure 8

Plasma glucose and insulin levels following STZ injection. Parameters were measured in blood collected at the time of sacrifice. Hyperglycemia and hypoinsulinemia were evident one week after diabetes induction and were sustained throughout the study. Values are expressed as mean ± SEM (n=8-10 animals). Stars represent significant differences from the control group (P<0.05) [Section 3.1].
WEEKS AFTER DIABETES INDUCTION

PLASMA GLUCOSE (mg/dl)
(BAR GRAPH)

PLASMA INSULIN (µU/ml)
(LINE GRAPH)

○ ○ control
■ ■ diabetic

WEEKS AFTER DIABETES INDUCTION
Plasma lipid profile in the experimental rats. Basal glycerol release from adipose tissue (Panel B), plasma triglycerides (Panel A) and plasma cholesterol (Panel C) were measured one, three and six weeks after diabetes induction. Values are expressed as mean ± SEM (n=8-10 animals). Stars represent significant differences from the control group (P < 0.05) [Section 3.1].

Figure 9
Figure 10

Effect of various left atrial filling pressures on the left ventricular developed pressure (LVDP) in hearts from control rats and rats diabetic for six weeks. Results are expressed as mean ± SEM; n, number of animals; asterisks denote significant differences from the control group (P < 0.05) [Section 3.1].
WISTAR

LVDP (mm Hg)

(○) CONTROL (n=7)
(◇) DIABETIC (n=8)

FILLING PRESSURE (Cm H₂O)
Table 2. Plasma glucose and insulin values of experimental rats.

<table>
<thead>
<tr>
<th></th>
<th>WISTAR</th>
<th>WISTAR-KYOTO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 8)</td>
<td>Diabetic (n = 8)</td>
</tr>
<tr>
<td></td>
<td>Control (n = 7)</td>
<td>Diabetic (n = 7)</td>
</tr>
<tr>
<td>Plasma Glucose</td>
<td>115 ± 2</td>
<td>333 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(mg/100 mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Insulin</td>
<td>42 ± 2</td>
<td>17 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(uU/mL)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The above parameters were measured in blood collected at the time of sacrifice. Results are expressed as mean ± SEM; n, number of animals.

<sup>a</sup> Significantly different from control of the same strain.
Figure 11

Plasma free fatty acids, total cholesterol, phospholipids and triglycerides in Wistar and Wistar-Kyoto rats 12 weeks after streptozotocin injection. Result is the % change from Wistar control. Each group consists of 6-8 animals [Section 3.2].
Basal glycerol release from adipose tissue of control and diabetic Wistar and Wistar-Kyoto rats. Glycerol was measured in the KRB buffer and is expressed as umoles per gm wet weight. Results are expressed as mean ± SEM; n, number of animals. * Significantly different from the other three groups (P<0.05). A Significantly different from Wistar control (P<0.05) [Section 3.2].
WISTAR CONTROL (N = 4)

WISTAR DIABETIC (N = 7)

WISTAR-KYOTO CONTROL (N = 5)

WISTAR-KYOTO DIABETIC (N = 5)
Figure 13

Myocardial triglyceride levels in hearts from Wistar and WKY control and diabetic rats 12 weeks after STZ injection. Results are the mean ± SEM for the number of hearts shown in parentheses. Asterisk indicates significant difference from Wistar control (P<0.05) [Section 3.2].
WISTAR CONTROL (n = 8)
WISTAR DIABETIC (n = 8)
WKY CONTROL (n = 7)
WKY DIABETIC (n = 7)
Effect of diabetes on +/- dP/dt of isolated perfused working hearts. Hearts were isolated from the 4 experimental groups 84 days after induction of diabetes. Values are mean ± SEM; n, number of hearts shown in parentheses. Data demonstrate the depression in heart function only in Wistar diabetic rats and were obtained at a left atrial filling pressure of 15 cm H₂O. *P<0.05 vs Wistar control [Section 3.2].
Wistar Control (n=8)
Wistar Diabetic (n=8)
WKY Control (n=7)
WKY Diabetic (n=7)
Table 3. Various parameters used to assess the diabetic state of hydralazine-treated rats.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th></th>
<th>STZ-INJECTED</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated (n = 8)</td>
<td>Hydralazine-Treated (n = 7)</td>
<td>Untreated (n = 7)</td>
<td>Hydralazine-Treated (n = 8)</td>
</tr>
<tr>
<td>Fluid Intake (mL/day)</td>
<td>78 ± 2</td>
<td>32 ± 1(^a)</td>
<td>268 ± 4(^{ab})</td>
<td>191 ± 4(^c)</td>
</tr>
<tr>
<td>Hydralazine Intake</td>
<td>0</td>
<td>50 ± 1.4</td>
<td>0</td>
<td>52 ± 1</td>
</tr>
<tr>
<td>(mg.Kg(^{-1}).day(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Glucose (mg/100 mL)</td>
<td>103 ± 14</td>
<td>135 ± 4</td>
<td>563 ± 17(^{ab})</td>
<td>514 ± 24(^{ab})</td>
</tr>
<tr>
<td>Plasma Triglycerides</td>
<td>103 ± 14</td>
<td>80 ± 11</td>
<td>446 ± 98(^c)</td>
<td>129 ± 10</td>
</tr>
<tr>
<td>(mg/100 mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Since diabetic rats drank more water than controls, the concentration of hydralazine had to be adjusted weekly to get a final dose between 50 and 55 mg.Kg\(^{-1}\).day\(^{-1}\) in both control and diabetic-treated groups. Results are expressed as mean ± SEM; n, number of animals.

\(^a\) Significantly different from control group
\(^b\) Significantly different from control-treated group
\(^c\) Significantly different from the other three groups (P < 0.05)
Figure 15

Basal glycerol release from adipose tissue of control and diabetic rats treated with hydralazine for six weeks. Glycerol was measured in the KRB buffer and is expressed as umole per gm wet weight. Results are mean ± SEM; n, number of animals. *Significantly different from the two control groups (P<0.05) [Section 3.3].
CONTROL (n=8)
DIABETIC (n=7)
CONTROL-TREATED (n=5)
DIABETIC-TREATED (n=5)

GLYCEROL OUTPUT (µmol/g/hour)
Figure 16

Effect of isoproterenol and hydralazine on glycerol release by adipose tissue from 6 week control and diabetic rats. For hydralazine, varying concentrations were used but only the highest concentration is indicated. A given adipose tissue fragment was exposed to only one concentration of hydralazine. For isoproterenol, dose response curves were performed but only the EC$_{50}$ value is indicated. To evaluate the effect of hydralazine on isoproterenol-stimulated lipolysis, adipose tissue fragments were first incubated with varying concentrations of hydralazine. Isoproterenol (EC$_{50}$) was then added to the incubation medium and glycerol release measured. Although varying concentrations of hydralazine were used, only the highest concentration is indicated. Results are the mean ± SEM for the number of experiments shown in parentheses [Section 3.3].
HYDRALAZINE $10^{-4}$M (N=5)
ISOPROTERENOL $10^{-7}$M (N=10)
ISOPROTERENOL ($10^{-7}$M) + HYDRALAZINE ($10^{-4}$M) (N=4)

BASAL (N=9)

CONTROL

DIABETIC

HYDRALAZINE $10^{-4}$M (N=5)
ISOPROTERENOL $10^{-7}$M (N=10)
ISOPROTERENOL ($10^{-7}$M) + HYDRALAZINE ($10^{-4}$M) (N=4)

BASAL (N=9)
Figure 17

Myocardial triglyceride levels in hearts from control and diabetic rats treated with hydralazine for six weeks. Results are the mean ± SEM for the number of hearts shown in parentheses. * Significantly different from the other three groups (P<0.05). ^ Significantly different from Wistar control (P<0.05) [Section 3.3].
CONTROL (N = 5)
DIABETIC (N = 7)
CONTROL TREATED (N = 4)
DIABETIC TREATED (N = 4)
Figure 18

Effect of diabetes and hydralazine treatment on LVDP of isolated perfused working hearts at various filling pressures. Hearts were isolated from the various groups 42 days after diabetes induction. Results are the mean ± SEM for 6-8 animals in each group. CON, control; COT, hydralazine-treated control; DIA, diabetic; DIT, hydralazine-treated diabetic [Section 3.3].
Table 4. General Features of the Experimental Rats: Pilot Study.\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>Sacrifice Body Weight (gms)</th>
<th>Ventricular Weight (gms)</th>
<th>Plasma Insulin (uU/mL)</th>
<th>Plasma Glucose (mg/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB-non diabetic littermates</td>
<td>404 ± 9</td>
<td>0.94 ± 0.01</td>
<td>63 ± 13</td>
<td>152 ± 10</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin-treated BB-diabetic</td>
<td>426 ± 7</td>
<td>1.20 ± 0.03</td>
<td>&gt; 160\textsuperscript{bc}</td>
<td>157 ± 13</td>
</tr>
<tr>
<td>(9 U.Kg\textsuperscript{-1}.day\textsuperscript{-1}) (n = 12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BB-diabetic; 2 days off insulin (n = 4)</td>
<td>375 ± 29</td>
<td>1.01 ± 0.03</td>
<td>37 ± 7</td>
<td>423 ± 7\textsuperscript{de}</td>
</tr>
<tr>
<td>BB-diabetic; 4 days off insulin (n = 19)</td>
<td>347 ± 13\textsuperscript{de}</td>
<td>0.89 ± 0.01\textsuperscript{ef}</td>
<td>29 ± 3</td>
<td>445 ± 28\textsuperscript{de}</td>
</tr>
</tbody>
</table>

Body weight was measured throughout the study period, but only the final values taken before sacrifice are indicated. The values for plasma insulin and glucose also represent those obtained in blood at sacrifice. Results are the mean ± SEM; n, number of animals.

\textsuperscript{a} Male BB-diabetic rats maintained for 2-5 weeks with insulin following the development of glycosuria

\textsuperscript{b} Significantly different from the other three groups

\textsuperscript{c} The range covered by the insulin RIA kit is 0-160 uU/mL

\textsuperscript{d} Significantly different from BB non-diabetic littermates

\textsuperscript{e} Significantly different from the insulin-treated group (P<0.05)
Figure 19

Time course of body weight changes in spontaneously diabetic BB rats upon withdrawal of insulin. Values are mean ± SEM of the 24 diabetic animals used for the pilot study [Section 3.4.1].
BODY WEIGHT (g)

DAYS OFF INSULIN
Effect of insulin withdrawal for 2 or 4 days on plasma triglycerides, total cholesterol and phospholipids of BB diabetic rats. These diabetic rats were maintained on 9 U.Kg\(^{-1}\). day\(^{-1}\) protamine zinc insulin (s.c.) for 2-5 weeks following development of glycosuria. Values are mean ± SEM; \( n \), number of animals. \(^A\) Significantly different from BB non-diabetic littermates (\( P<0.05 \)); \(^B\) Significantly different from insulin-treated BB diabetic rats given 9 U.Kg\(^{-1}\).day\(^{-1}\) protamine zinc insulin (\( P<0.05 \)) [Section 3.4.1].
110

CD BB NON-DIABETIC LITTER MATES (n=10)
INSULIN-TREATED BB DIABETIC 9U/Kg/DAY PZI (n=12)
BB DIABETIC; TWO DAYS OFF INSULIN (n=4)
BB DIABETIC; FOUR DAYS OFF INSULIN (n=19)

PLASMA TRIGLYCERIDES
PLASMA CHOLESTEROL
PLASMA PHOSPHOLIPIDS

(mg/dL)
<table>
<thead>
<tr>
<th></th>
<th>Wistar Control</th>
<th>BB Non-diabetic Littermates</th>
<th>BB Diabetic High Dose Insulin</th>
<th>BB Diabetic Low Dose Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sacrifice Body Weight (gms)</td>
<td>483 ± 24 (10)</td>
<td>462 ± 8 (10)</td>
<td>472 ± 28 (6)</td>
<td>421 ± 18 (12)</td>
</tr>
<tr>
<td>Ventricular Weight (mg)</td>
<td>1196 ± 46 (4)</td>
<td>987 ± 17 (4)</td>
<td>1061 ± 49 (4)</td>
<td>810 ± 99a (4)</td>
</tr>
<tr>
<td>Plasma Insulin (uU/mL)</td>
<td>72 ± 17 (10)</td>
<td>52 ± 6 (10)</td>
<td>112 ± 28bc (6)</td>
<td>62 ± 6 (12)</td>
</tr>
<tr>
<td>Plasma Glucose (mg/ 100 mL)</td>
<td>129 ± 9 (10)</td>
<td>123 ± 4 (10)</td>
<td>137 ± 42 (6)</td>
<td>275 ± 52d (12)</td>
</tr>
<tr>
<td>Plasma T3 (nM/L)</td>
<td>1.13 ± 0.05 (10)</td>
<td>1.2 ± 0.08 (10)</td>
<td>1.0 ± 0.16 (6)</td>
<td>1.17 ± 0.13 (12)</td>
</tr>
<tr>
<td>Plasma T4 (nM/L)</td>
<td>47 ± 2 (10)</td>
<td>47 ± 2 (10)</td>
<td>42 ± 3 (6)</td>
<td>44 ± 4 (12)</td>
</tr>
</tbody>
</table>

Body weight was measured throughout the study period, but only the final values taken before sacrifice are indicated. Parameters were measured in blood collected at the time of sacrifice. Results are the mean ± SEM for the number of animals shown in parentheses.

a Significantly different from Wistar control
b Significantly different from BB-diabetic low dose insulin
c Significantly different from BB non-diabetic littermates
d Significantly different from the other three groups (P < 0.05)
Response of BB diabetic rats to low and high dose insulin treatment 4, 8 and 12 weeks after the treatment was initiated. Values are mean ± SEM; n, number of animals. *Significantly different from BB diabetic group treated with low-dose insulin (P<0.05) [Section 3.4.2].
WEEKS AFTER INSULIN TREATMENT

A. BB DIABETIC LOW-DOSE INSULIN (n=6)
B. BB DIABETIC HIGH-DOSE INSULIN (n=12)
Figure 22

Plasma triglycerides, cholesterol and phospholipids of the various groups determined in blood collected at the time of sacrifice. Values are mean ± SEM; n, number of animals. *Significantly different from Wistar control (P<0.05); ^Significantly different from BB diabetic group treated with high-dose insulin (P<0.05) [Section 3.4.2].
CD WISTAR CONTROL (n=10)
BB NON-DIABETIC LITTER MATES (n=10)
BB DIABETIC HIGH-DOSE INSULIN (n=6)
BB DIABETIC LOW-DOSE INSULIN (n=12)

PLASMA PLASMA PLASMA
TRIGLYCERIDES CHOLESTEROL PHOSPHOLIPIDS

(mg/dL)

250
200
150
100
50
0

PLASMA TRIGLYCERIDES
PLASMA CHOLESTEROL
PLASMA PHOSPHOLIPIDS
Figure 23

ATP-dependent Ca\(^{2+}\) uptake in cardiac sarcoplasmic reticulum. Ca\(^{2+}\) uptake was measured at various free Ca\(^{2+}\) concentrations between 0.1 and 2.0 umole. Hearts were isolated from the various groups 84 days after the development of glycosuria. Values are the mean ± SEM for the number of hearts shown in parentheses [Section 3.4.2].
CALCIUM UPTAKE (nmol Ca$^{2+}$/mg/min)

0.0 0.5 1.0 1.5 2.0
Ca$^{2+}$ (uM)

- WISTAR CONTROL (n=4)
- BB NON-DIABETIC LITTERMATES (n=4)
- BB DIABETIC HIGH-DOSE INSULIN (n=4)
- BB DIABETIC LOW-DOSE INSULIN (n=4)
Figure 24

Effect of low-dose insulin treatment on (A) LVDP, (B) -dP/dt and (C) +dP/dt of isolated perfused working hearts at various filling pressures. Hearts were isolated from different groups 84 days after the development of glycosuria. Each value is the mean ± SEM for the number of hearts shown in parentheses [Section 3.4.2].
FILLING PRESSURE (cm H2O)

A. BB NON-DIABETIC LITTERMATES (n=6)
B. BB DIABETIC LOW-DOSE INSULIN (n=6)
C. WISTAR CONTROL (n=6)
Table 6. General features of the experimental rats and various parameters used to assess the diabetic state.

<table>
<thead>
<tr>
<th></th>
<th>Wistar Control (n = 12)</th>
<th>BB Non-diabetic Littermates (n = 6)</th>
<th>BB-diabetic (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sacrifice Body Weight (gms)</td>
<td>541 ± 8</td>
<td>500 ± 9\textsuperscript{a}</td>
<td>434 ± 21\textsuperscript{ab}</td>
</tr>
<tr>
<td>Plasma Insulin (uU/mL)</td>
<td>82 ± 9</td>
<td>35 ± 2\textsuperscript{a}</td>
<td>31 ± 5\textsuperscript{a}</td>
</tr>
<tr>
<td>Plasma Glucose (mg/100 mL)</td>
<td>116 ± 3</td>
<td>123 ± 3</td>
<td>317 ± 24\textsuperscript{ab}</td>
</tr>
<tr>
<td>Plasma T\textsubscript{4} (nM/L)</td>
<td>46 ± 2</td>
<td>41 ± 2</td>
<td>34 ± 4\textsuperscript{a}</td>
</tr>
<tr>
<td>Ca\textsuperscript{2+}-ATPase of Myosin (uM P\textsubscript{i}-mg protein\textsuperscript{-1}.min\textsuperscript{-1})</td>
<td>-</td>
<td>1.25 ± 0.08</td>
<td>0.868 ± 0.1\textsuperscript{b}</td>
</tr>
</tbody>
</table>

Body weight was measured throughout the study period, but only the final values taken before sacrifice are indicated. Parameters were measured in blood collected at the time of sacrifice. Results are the mean ± SEM; \( n \), number of animals. The BB diabetic rats were treated with 3.5 U.Kg\textsuperscript{-1}.day\textsuperscript{-1} protamine zinc insulin.

\( \text{a} \) Significantly different from Wistar control  
\( \text{b} \) Significantly different from BB non-diabetic littermates  
\( P < 0.05 \)
Plasma triglycerides and total cholesterol of the various groups determined in blood collected at the time of sacrifice. Values are mean ± SEM. *Significantly different from Wistar control (P<0.05); ‖Significantly different from BB non-diabetic littermates (P<0.05) [Section 3.4.3].
A. PLASMA TRIGLYCERIDES (mg/dL)  

- CONTROL
- BB NON-DIABETIC LITTERMATES
- BB DIABETIC (3.5 U/Kg/Day)

B. PLASMA CHOLESTEROL (mg/dL)
Figure 26

Effect of diabetes on (A) $+\frac{dP}{dt}$, (B) $-\frac{dP}{dt}$ and (C) LVDP of isolated perfused working hearts at various filing pressures. Hearts were isolated from the 2 groups 6 weeks after the development of glycosuria. Values shown are mean ± SEM. Asterisks indicate significant difference from BB non-diabetic littermates ($P<0.05$) [Section 3.4.3].
FILLING PRESSURE (cm H20)

- BB Diabetic (3.5 U/Kg/Day)
- BB Non-Diabetic Littermates
Table 7. General features of the experimental rats.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>STZ-INJECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated (n = 8)</td>
<td>Untreated (n = 8)</td>
</tr>
<tr>
<td></td>
<td>Carnitine Treated (n = 6)</td>
<td>Carnitine Treated (n = 8)</td>
</tr>
<tr>
<td>Body Weight (gms)</td>
<td>400 ± 9</td>
<td>298 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>296 ± 12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fluid Intake (mL/day)</td>
<td>41 ± 0.8</td>
<td>123 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>56 ± 2</td>
</tr>
<tr>
<td>Wet Heart Weight (gms)</td>
<td>1.64 ± 0.1</td>
<td>1.06 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.22 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Body weight was measured throughout the 6 week period, but only the final values taken before sacrifice are indicated. Results are expressed as mean ± SEM; n, number of animals.

a Significantly different from untreated control
b Significantly different from the other three groups (P<0.05)
Table 8. Various indices used to assess the diabetic state of experimental rats.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL Untreated (n = 8)</th>
<th>CONTROL Carnitine Treated (n = 6)</th>
<th>STZ-INJECTED Untreated (n = 8)</th>
<th>STZ-INJECTED Carnitine Treated (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Insulin (uU/mL)</td>
<td>55 ± 6</td>
<td>47 ± 7</td>
<td>20 ± 2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>23 ± 2&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma T&lt;sub&gt;3&lt;/sub&gt; (nM/L)</td>
<td>0.74 ± 0.05</td>
<td>0.68 ± 0.05</td>
<td>0.49 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.66 ± 0.04</td>
</tr>
<tr>
<td>Plasma T&lt;sub&gt;4&lt;/sub&gt; (nM/L)</td>
<td>57 ± 5</td>
<td>48 ± 4</td>
<td>30 ± 4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>43 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Myocardial free Carnitine (nM/gm dry weight)</td>
<td>287 ± 37</td>
<td>297 ± 37</td>
<td>131 ± 25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>324 ± 28</td>
</tr>
</tbody>
</table>

Indices were measured in blood collected at the time of death. For the insulin assay, human insulin standards were used because error made in the estimation of rat insulin was minimal owing to the very high cross-reactivity with rat insulin (about 90%). Results are expressed as mean ± SEM; n, number of animals.

a Significantly different from untreated control
b Significantly different from L-carnitine treated control
c Significantly different from the other three groups (P<0.05)
Figure 27

Time course of body weight increases in control and diabetic rats. Body weights were measured daily prior to injecting L-carnitine. Values are means ± SEM; n, number of animals; ○, control treated with saline; ▼, diabetic treated with saline; ▲, control treated with L-carnitine; ●, diabetic treated with L-carnitine; § significantly different from other 3 groups (P<0.05) [Section 3.5.1].
(○) CON (saline), n=8
(▲) CON-T (L-carnitine), n=6
(●) DIA-T (L-carnitine), n=8
(▼) DIA (saline), n=8

WEEKS AFTER DIABETES INDUCTION
Figure 28

Concentration of glucose in plasma measured six weeks after diabetes induction with STZ. Results are mean ± SEM for number of animals shown in parentheses. CON, control; DIA, diabetic; T, treated. *Significantly different from untreated controls; †Significantly different from L-carnitine treated controls; §Significantly different from the other three groups (P<0.05) [Section 3.5.1].
• CON (saline) (n=8)
• CON-T (L-carnitine) (n=6)
• DIA-T (L-carnitine) (n=8)
• DIA (saline) (n=8)

PLASMA GLUCOSE (mg/dl)
Effect of diabetes and L-carnitine treatment on LVDP of isolated perfused working hearts at various filling pressures. Hearts were isolated from experimental groups six weeks after induction of diabetes with STZ. Each point represents mean ± SEM for number of hearts shown in parentheses. CON, control; DIA, diabetic; T, treated. Significantly different from the other three groups (P < 0.05) [Section 3.5.1].
Effect of diabetes and L-carnitine treatment on $+\frac{dP}{dt}$ of isolated perfused working hearts at various filling pressures. Hearts were isolated from experimental groups six weeks after diabetes induction. Each point represents mean ± SEM for number of hearts shown in parentheses. CON, control; DIA, diabetic; T, treated. §Significantly different from the other three groups (P < 0.05) [Section 3.5.1].
FILLING PRESSURE (cm H₂O)

- +dP/dt (mm Hg/s)

- ● CON (saline), n=8
- ■ CON-T (L-carnitine), n=6
- ▲ DIA-T (L-carnitine), n=8
- ○ DIA (saline), n=8
Effect of diabetes and L-carnitine treatment on -dp/dt of isolated perfused working hearts at various filling pressures. Hearts were isolated from experimental groups six weeks after diabetes induction. Each point represents mean ± SEM for number of hearts shown in parentheses. CON, control; DIA, diabetic; T, treated. $^8$Significantly different from the other three groups (P<0.05) [Section 3.5.1].
-dP/dt (mm Hg/s)

FILLING PRESSURE (cm H₂O)

- CON (saline), n=8
- CON-T (L-carnitine), n=6
- DIA-T (L-carnitine), n=8
- DIA (saline), n=8
Effect of diabetes and L-carnitine treatment on plasma triglycerides, total cholesterol and phospholipids of the experimental rats. Each point is mean ± SEM for the number of animals shown in parentheses. CON, control; DIA, diabetic; T, treated. § Significantly different from the other three groups (P < 0.05) [Section 3.5.1].
Table 9. Effect of diabetes and L-carnitine treatment (3 g.Kg$^{-1}$.day$^{-1}$) on various indices 8 weeks after diabetes induction.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL (n = 6)</th>
<th>STZ-INJECTED (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>Carnitine Treated</td>
</tr>
<tr>
<td>Plasma Glucose</td>
<td>122 ± 4</td>
<td>114 ± 3</td>
</tr>
<tr>
<td>(mg/100 mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Triglyceride</td>
<td>112 ± 21</td>
<td>64 ± 5</td>
</tr>
<tr>
<td>(mg/100 mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Cholesterol</td>
<td>46 ± 4</td>
<td>46 ± 4</td>
</tr>
<tr>
<td>(mg/100 mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Phospholipid</td>
<td>125 ± 9</td>
<td>110 ± 5</td>
</tr>
<tr>
<td>(mg/100 mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal Glycerol</td>
<td>65 ± 7</td>
<td>74 ± 8</td>
</tr>
<tr>
<td>Output (uM.gm$^{-1}$.hr$^{-1}$)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

L-carnitine treatment was begun 6 weeks after the development of glycosuria and was continued for a 2 week period. Results are expressed as mean ± SEM; n, number of animals.

a Significantly different from treated control
b Significantly different from untreated control
c Significantly different from the other three groups
(P < 0.05)
Figure 33

Time course of body weight increases in various groups of rats used for the reversal study. Weights were measured daily prior to injecting L-carnitine. Values are mean weights of all animals (6-7) in each group. CON, saline-treated control; COT, L-carnitine-treated control; DIA, saline-treated diabetic; DIT, L-carnitine-treated diabetic. To simplify the figure, SE bars are not drawn. * Significantly different from CON and COT (P<0.05); † Significantly different from CON (P<0.05) [Section 3.5.2].
Figure 34

Fluid (Panel A) and food (Panel B) intake in various groups of rats used for the reversal study. The values represent the mean of all animals (6-7) in each group. To simplify the figure, SE bars are not drawn. L-carnitine treatment (3 g.Kg$^{-1}$.day$^{-1}$) was started six weeks after STZ injection and continued for two weeks [Section 3.5.2].
Effect of diabetes and L-carnitine treatment on LVDP of isolated perfused working hearts at various filling pressures. Hearts were isolated from 4 experimental groups eight weeks after diabetes induction and two weeks after L-carnitine treatment. Each point represents mean ± SEM for 6-7 hearts. Asterisks represent significant differences from the two control groups (P<0.05) [Section 3.5.2].
Table 10. General features of experimental rats used for the low dose (0.5 g.Kg\(^{-1}\).day\(^{-1}\)) L-carnitine study.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL (n = 10)</th>
<th>STZ-INJECTED Untreated (n = 9)</th>
<th>Carnitine Treated (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (gms)</td>
<td>464 ± 11</td>
<td>361 ± 10(^a)</td>
<td>354 ± 9(^a)</td>
</tr>
<tr>
<td>Food Intake (gm/day)</td>
<td>32 ± 1</td>
<td>52 ± 1(^a)</td>
<td>40 ± 2(^{ab})</td>
</tr>
<tr>
<td>Fluid Intake (mL/day)</td>
<td>46 ± 2</td>
<td>247 ± 27(^a)</td>
<td>175 ± 20(^{ab})</td>
</tr>
</tbody>
</table>

These indices were measured throughout the six week period, but only the final values taken before death are indicated. Results are expressed as mean ± SEM; n, number of animals.

\(^a\) Significantly different from control
\(^b\) Significantly different from untreated diabetic (P<0.05)
Tables 11. Various indices used to assess the diabetic state of experimental rats used for the low dose (0.5 g.Kg\(^{-1}\).day\(^{-1}\)) L-carnitine study.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL (n = 10)</th>
<th>STZ-INJECTED Untreated (n = 9)</th>
<th>Carnitine Treated (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Glucose</td>
<td>122 ± 4</td>
<td>449 ± 15(^a)</td>
<td>269 ± 41(^{ab})</td>
</tr>
<tr>
<td>(mg/100 mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Triglycerides</td>
<td>112 ± 19</td>
<td>185 ± 8(^c)</td>
<td>126 ± 13</td>
</tr>
<tr>
<td>(mg/100 mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Phospholipids</td>
<td>125 ± 9</td>
<td>172 ± 7(^c)</td>
<td>141 ± 4</td>
</tr>
<tr>
<td>(mg/100 mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Cholesterol</td>
<td>46 ± 3(^c)</td>
<td>66 ± 4</td>
<td>58 ± 2</td>
</tr>
<tr>
<td>(mg/100 mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma T(_3)</td>
<td>1.2 ± 0.06</td>
<td>0.57 ± 0.06(^a)</td>
<td>0.86 ± 0.1(^{ab})</td>
</tr>
<tr>
<td>(nM/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca(^{2+}) -ATPase of Myosin</td>
<td>1.8 ± 0.1(^c)</td>
<td>0.96 ± 0.09</td>
<td>1.27 ± 0.02</td>
</tr>
<tr>
<td>(uM P(_{i}).mg(^{-1}) protein.min(^{-1}))</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Indices were measured in blood collected at the time of sacrifice, 6 weeks after diabetes induction with STZ. Ca\(^{2+}\) -ATPase of myosin was measured in hearts frozen at the end of a function curve. Results are expressed as mean ± SEM; n, number of animals.

\(^a\) Significantly different from control
\(^b\) Significantly different from untreated diabetic
\(^c\) Significantly different from the other two groups (P<0.05)
Effect of diabetes and L-carnitine treatment on (A) LVDP, and (B) +dP/dt of isolated perfused working hearts at various filling pressures. Hearts were isolated from control (●-●), diabetic (▲-▲), and carnitine-treated diabetic (■-■) rats six weeks after induction of diabetes. Carnitine-treated diabetic rats received a daily dose of 0.5 g.Kg$^{-1}$.day$^{-1}$ L-carnitine (i.p.) throughout the study period. The dosing commenced 3 days after induction of diabetes. Each point is the mean ± SEM of 9-10 animals in each group. Asterisks indicate significant differences from CON and DIT (P<0.05) [Section 3.5.3].
Heart function in control and diabetic rats, perfused with L-carnitine, 6 weeks after STZ injection [Section 3.5.4].

A. Effect of perfusion time on LVDP. Hearts were perfused at a fixed filling pressure of 15 cm H$_2$O. Varying concentrations of L-carnitine were used but only the highest concentration perfused is indicated. A given heart was exposed to only one concentration of L-carnitine. Asterisks indicate significant differences from control (P < 0.05).

B. Heart function (LVDP) in control and diabetic rats at various left atrial filling pressures. After equilibration, the perfusion medium was switched to CK buffer containing varying concentrations of L-carnitine up to 1 mM and function curves were performed as described in the section 2.6. During the function curve, a given heart was exposed to only one concentration of L-carnitine. Asterisks indicate significant differences from control (P < 0.05).
A. **FILLING PRESSURE (cm H2O)**

![Diagram A](image)

B. **LVDP (mmHg)**

![Diagram B](image)
Table 12. Effect of oral administration of L-carnitine in 1 week diabetic rats.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>DIABETIC L-Carnitine (mg.Kg⁻¹.day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Plasma Acyl/Total Carnitine</td>
<td>0.18 ± 0.05</td>
<td>0.44 ± 0.04</td>
</tr>
<tr>
<td>Plasma Triglycerides (mg/100 mL)</td>
<td>119 ± 12</td>
<td>183 ± 15</td>
</tr>
<tr>
<td>Plasma Cholesterol (mg/100 mL)</td>
<td>55 ± 1</td>
<td>68 ± 2</td>
</tr>
<tr>
<td>Plasma Glucose (mg/100 mL)</td>
<td>134 ± 4</td>
<td>393 ± 12</td>
</tr>
<tr>
<td>Plasma T₄ (nM/L)</td>
<td>54 ± 4</td>
<td>33 ± 3</td>
</tr>
<tr>
<td>Total Myocardial Carnitine (nM.gm⁻¹ dry weight)</td>
<td>4730 ± 337</td>
<td>3335 ± 501</td>
</tr>
</tbody>
</table>

Plasma indices were measured in blood collected at the time of sacrifice. Results are expressed as mean ± SEM for 4-6 animals in each group.
Table 13. Effect of oral administration of L-carnitine in 3 weeks diabetic rats.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>DIABETIC L-Carnitine (mg.Kg⁻¹.day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Plasma Acyl/Total</td>
<td>0.26</td>
<td>0.60</td>
</tr>
<tr>
<td>Carnitine</td>
<td>± 0.08</td>
<td>± 0.02</td>
</tr>
<tr>
<td>Plasma Triglycerides</td>
<td>93 ± 9</td>
<td>224 ± 29</td>
</tr>
<tr>
<td>(mg/100 mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Cholesterol</td>
<td>68 ± 2</td>
<td>110 ± 13</td>
</tr>
<tr>
<td>(mg/100 mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Glucose</td>
<td>136 ± 1</td>
<td>473 ± 18</td>
</tr>
<tr>
<td>(mg/100 mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma T₄</td>
<td>99 ± 7</td>
<td>38 ± 4</td>
</tr>
<tr>
<td>(nM/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Myocardial</td>
<td>4517</td>
<td>2450</td>
</tr>
<tr>
<td>Carnitine</td>
<td>± 406</td>
<td>± 187</td>
</tr>
<tr>
<td>(nM.gm⁻¹ dry weight)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Plasma indices were measured in blood collected at the time of sacrifice. Results are expressed as mean ± SEM for 4-6 animals in each group.
Table 14. Various indices used to assess the diabetic state of control and diabetic rats after oral administration of L-carnitine for 6 weeks.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL Untreated</th>
<th>CONTROL Carnitine Treated</th>
<th>STZ-INJECTED Untreated</th>
<th>STZ-INJECTED Carnitine Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. General Features</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sacrifice Body Weight (gms)</td>
<td>464 ± 11</td>
<td>468 ± 13</td>
<td>359 ± 9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>342 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fluid Intake (mL/day)</td>
<td>46 ± 2</td>
<td>52 ± 3</td>
<td>297 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>280 ± 21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Food Intake (gm/day)</td>
<td>32 ± 1</td>
<td>32 ± 1</td>
<td>56 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>II. Plasma Indices</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol (mg/100 mL)</td>
<td>63 ± 3</td>
<td>62 ± 2</td>
<td>84 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglycerides (mg/100 mL)</td>
<td>148 ± 12</td>
<td>112 ± 10</td>
<td>313 ± 35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>242 ± 50</td>
</tr>
<tr>
<td>Phospholipids (mg/100 mL)</td>
<td>122 ± 4</td>
<td>118 ± 4</td>
<td>228 ± 34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>164 ± 13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose (mg/100 mL)</td>
<td>133 ± 3</td>
<td>130 ± 3</td>
<td>415 ± 13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>466 ± 17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Insulin (uM/L)</td>
<td>71 ± 4</td>
<td>86 ± 12</td>
<td>32 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt; (nM/L)</td>
<td>46 ± 2</td>
<td>42 ± 2</td>
<td>25 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Indices were measured in blood collected at the time of death. Results are expressed as mean ± SEM for 10-12 animals in each group.

<sup>a</sup> Significantly different from the two control groups (P < 0.05)
Figure 38

Time course of body weight increases in control and diabetic rats. Body weights were measured daily. After the third day of diabetes induction, the rats received various oral doses of L-carnitine during the study period. Results are the means of 4-6 animals in each group [Section 3.5.5].
BODY WEIGHT

L-Carnitine Oral Treatment
Pilot Study
Figure 39

Plasma carnitine levels (total, free and acyl) from one week control and STZ-diabetic rats. The diabetic rats received various oral doses of L-carnitine (50, 150, 250 mg.Kg$^{-1}$.day$^{-1}$) throughout the study period. Results are expressed as means ± SEM for 4-6 animals in each group. CON, control; DIA, diabetic [Section 3.5.5].
**TOTAL CARNITINE**

- **uM/L**
- **CON DIA 50 150 250**

**FREE CARNITINE**

- **uM/L**
- **CON DIA 50 150 250**

**ACYL CARNITINE**

- **uM/L**
- **CON DIA 50 150 250**

**L-CARNITINE DIABETIC**

- **(mg/Kg/Day)**
Plasma carnitine levels (total, free and acyl) from three week control and STZ-diabetic rats. The diabetic rats received various oral doses of L-carnitine (50, 150, 250 mg.Kg$^{-1}$.day$^{-1}$) throughout the study period. Results are expressed as means ± SEM for 4-6 animals in each group. CON, control; DIA, diabetic [Section 3.5.5].
Figure 41

Effects of L-carnitine treatment on the levels of total, free and acyl carnitine in the plasma of control and diabetic rats. Rats received L-carnitine (200 mg.Kg⁻¹.day⁻¹) in their drinking water for six weeks. Values represent mean ± SEM for 10-12 animals in each group. a Significantly different from the two control groups (P<0.05) [Section 3.5.5].
TOTAL CARNITINE

FREE CARNITINE

ACYL CARNITINE
Effect of diabetes and oral L-carnitine treatment (200 mg.Kg\(^{-1}\).day\(^{-1}\)) on (A) \(+dP/dt\), (B) \(-dP/dt\) and (C) LVDP of isolated perfused working hearts at various filling pressures. Hearts were isolated from the four groups six weeks after the induction of diabetes. Values represent the mean ± SEM for 10-12 hearts in each group [Section 3.5.5].
FILLING PRESSURE (cm H₂O)

- • DIABETIC
- ▲ DIABETIC TREATED
- ● CONTROL
- ○ CONTROL TREATED
DISCUSSION

Rats treated with STZ exhibited the classical features of Type 1 diabetes (IDDM). Quantitative determination of various physiological parameters revealed hyperglycemia, hypoinsulinemia, hyperlipidemia, polyphagia and polydipsia in diabetic rats which also weighed less than controls (Figures 7-9). Qualitative observations confirmed the presence of polyuria and glycosuria. All of these symptoms of diabetes have been reported in a number of previous studies with STZ (Fein et al. 1980, 1981; Vadlamudi et al. 1982; Rodrigues and McNeill 1986). However, it cannot be conclusively stated that the STZ-diabetic animals represent Type 1 diabetes in humans. This is because these animals have reduced (by 50%) but still significant amounts of circulating insulin and can survive the disease without administration of exogenous insulin. On the other hand, if left untreated, the rats develop a number of complications which are seen clinically during diabetes. For example, isolated perfused working hearts from STZ-diabetic Wistar rats exhibited a decreased ability to develop peak left ventricular pressure when exposed to high work loads (Figure 10). A decrease in maximum +/- dP/dt of left ventricle in these rats has also been previously reported (Vadlamudi et al. 1982; Rodrigues et al. 1986). Since the vascular (Reinila 1981) and nervous (Bestelli et al. 1981) systems are also affected, it would be reasonable to refer to these rats as poorly controlled Type 1 diabetics.

The pathogenesis of the myocardial defects seen during diabetes is complex. These defects may include a variety of cardiovascular conditions involving depressed myosin and actomyosin Ca$^{2+}$-ATPase (Malhotra et al. 1981), depressed SR Ca$^{2+}$-Mg$^{2+}$-ATPase activities (Lopaschuk et al. 1983; Ganguly et al. 1983) and a defective sarcolemmal Ca$^{2+}$ pump (Heyliger et al. 1987). However, the metabolic basis of these myocardial defects is unclear. They may be related to the inability of the diabetic heart to utilize glucose as an energy substrate (Miller 1979) and hence its
exclusive dependence on fatty acid for energy production (Randle et al. 1984). When this occurs, the high lipid levels in the plasma, or elevated lipid intermediates in the heart, are thought to be potentially noxious to the myocardium. They interfere with various cellular functions by specifically inhibiting enzymes or non-specifically altering the structure of membranes by their detergent like effects (Vaartjes et al. 1972; Helenius and Simmons 1975; Katz and Messineo 1981; Corr et al. 1982). Indeed, the present experiments with WKY diabetic rats or hydralazine-treated diabetic rats suggested a strong correlation between myocardial alterations and lipid abnormalities.

In the WKY study, plasma glucose was found to be consistently high in all rats (Wistar and WKY) receiving the diabetogen with corresponding hypoinsulinemia (Table 2). However, even at similar degrees of hyperglycemia and hypoinsulinemia, WKY diabetic rats did not show elevated plasma (Figure 11) or myocardial lipids (Figure 13) while the Wistar diabetic group did. The reason why the plasma and myocardial lipid levels are not elevated in WKY diabetic rats despite their insulin deficiency is obscure. It may be due to the fact that basal glycerol output from adipose tissue in these rats is low relative to Wistar diabetic animals (Figure 12). Irrespective of the mechanism, the results are certainly important because the lack of cardiac dysfunction in WKY diabetic rats (Figure 14) could be explained on the basis of their normal lipid levels.

Similar conclusions were obtained in the hydralazine study. The data for plasma (Table 3) and myocardial triglycerides (Figure 17) in the untreated STZ-diabetic Wistar rats were in agreement with previous reports (Nikkila 1974; Brunzell et al. 1979; Heyliger et al. 1986). However, hydralazine-treatment reduced these elevated plasma or cardiac triglyceride levels (Table 3, Figure 17). The pronounced effect of this antihypertensive agent in lowering blood lipids in STZ-diabetic Wistar rats was surprising but not totally unexpected. In human patients, other authors have also reported this unusual ability of hydralazine to lower blood lipids (Perry and Schroeder
1955; Deming et al. 1958; Perry and Mills 1962). A previous study has shown that this effect of hydralazine on lipid levels of diabetic rats appeared to be independent of insulin levels (Rodrigues et al. 1986).

It is not clear why lipid levels are not elevated in these hydralazine-treated diabetic rats, even when they lack insulin. Lipolytic hormones activate adenylate cyclase to stimulate cAMP accumulation in the adipocyte. They also enhance Ca\(^{2+}\)-uptake in the adipocyte. By these mechanisms, they activate cAMP dependent protein kinases which are capable of phosphorylating substrate proteins, including hormone-sensitive lipase (Kawai 1985). The phosphorylation and activation of triglyceride lipase results in the degradation of stored triglyceride to fatty acid and glycerol. In contrast, the antilipolytic hormone insulin decreases cAMP accumulation and Ca\(^{2+}\)-uptake into the adipocyte (Kawai 1985). It could be speculated that hydralazine, like insulin, inhibits lipolysis by affecting Ca\(^{2+}\) fluxes or by decreasing intracellular cAMP. To determine whether or not the mechanism of action of hydralazine as a lipid lowering agent occurred at the site of adipose tissue, the \textit{in vivo} and \textit{in vitro} effects of hydralazine were tested in adipose tissue from control and diabetic rats. The results indicated that hydralazine, both \textit{in vivo} and \textit{in vitro}, had no effect on the basal release of glycerol from adipose tissue (Figures 15 and 16). The release rates remained elevated in diabetic rats.

Another possible explanation for the decrease in plasma lipids is that the metabolic changes observed in untreated diabetes are, in many respects, similar to those produced by infusion of catecholamines. They include hyperglycemia, decreased glucose tolerance, elevated free-fatty acids and ketone bodies in the plasma. Catecholamines bind to beta-receptors of fat cells and increase cAMP which, in turn, leads to activation of triglyceride lipase. This causes the degradation of stored triglyceride to fatty acid and glycerol. Indeed, isoproterenol, used as the lipolytic stimulus, increased glycerol output from both control and diabetic rats adipose tissue.
Elevated plasma catecholamine levels have been reported in experimental and clinical diabetes (Christensen, 1974; Ganguly et al. 1986). Thus, the absence of elevated plasma lipids in hydralazine-treated diabetic rats could be due to a direct effect of hydralazine on the synthesis and release of catecholamines. Hydralazine has been shown to inhibit dopa-decarboxylase (Sano et al., 1960) and dopamine beta-hydroxylase in an extract prepared from bovine adrenal glands (Liu et al. 1974). It also produces a concentration-dependent inhibition of the conversion of \[^{3}H\]dopamine to \[^{3}H\]noradrenaline in isolated or intact rat atria (Songittiguna et al. 1980). Chevillard et al. (1980) have shown that hydralazine superfusion of isolated proximal segments of rat tail artery inhibited vasoconstrictor responses to transmural stimulation in a concentration-dependent manner. Hydralazine is also known to inhibit the spontaneous release of noradrenaline in rat heart (Songittiguna and Rand 1982).

However, in this study, hydralazine (10\(^{-8}\) to 10\(^{-4}\)M) did not reduce the isoproterenol induced glycerol release at any concentration (Figure 16). Similar results have been reported in isolated fat cells from normal rats and dogs (Grund and McNeill 1989). Hence, it could be concluded that the lipid lowering effect of hydralazine does not occur at the site of adipose tissue free fatty acid mobilization. Whatever the mechanism of hydralazine in lowering lipid levels, the function of hearts in hydralazine-treated diabetic rats was not different from that in non-diabetic controls (Figure 18). In keeping with the WKY study, it would appear that elevated plasma and myocardial lipids are important determinants of cardiac dysfunction in diabetes.

The results from the above studies were obtained in STZ-induced diabetic rats. However, as mentioned in section 1.3.1., there remain a few problems with this model. Hence, it was decided to study metabolic alterations and cardiac functional changes in the spontaneously diabetic BB rat. In a pilot study, it was apparent that the spontaneously diabetic BB rat, as a model of diabetes, has many similarities to IDDM. This observation is consistent with previous reports (Chappel and Chappel 1983;
Marliss et al. 1982). The rats were insulin-dependent and, upon insulin withdrawal, widespread metabolic changes such as loss of body weight (Figure 19), hyperglycemia (Table 4) and hyperlipidemia (Figure 20) occurred. It was thus hypothesized that rats with this spontaneous form of diabetes would develop diabetic cardiomyopathy similar to that seen in human diabetes mellitus (Kannel and McGee 1979) or in the diabetic state produced by injecting STZ or alloxan (Fein et al. 1981; Vadlamudi et al. 1982; Tahiliani et al. 1983).

Alterations that occur in the heart as a result of diabetic cardiomyopathy have been found to involve pronounced changes in left ventricular functions of which a prominent feature is the slowing of the rate at which the affected ventricular muscle can contract and relax (Fein et al. 1980; Vadlamudi et al. 1982). These alterations in the rate of contraction and relaxation could occur as a result of a defect in cardiac cell 

\[ \text{Ca}^{2+} \]\n
handling. Malhotra et al. (1981, 1985) have examined contractile protein function in preparations from STZ-diabetic and BB diabetic rats and found depressed 

\[ \text{Ca}^{2+} \]\n
-activated myosin and actomyosin ATPase activities. These findings could explain abnormal cardiac contraction but not the slowing of relaxation. This latter phenomenon may reflect a defect within the diabetic myocardial SR.

Cardiac SR membranes contain a 

\[ \text{Ca}^{2+} \]\n
-dependent ATPase enzyme (MacLennan 1970) which transports 

\[ \text{Ca}^{2+} \]\n
from the sarcoplasm into the SR vesicles with a high velocity and affinity. Consequently, this promotes relaxation of the myofibrils (Reuter 1974). It also probably acts as an intracellular store of activator 

\[ \text{Ca}^{2+} \]\n
, the release of which activates the contractile mechanism following cardiac cell depolarization. Any alteration in the ability of the SR to transport 

\[ \text{Ca}^{2+} \]\n
would therefore be expected to have an important impact on the contractile performance of the heart. Indeed, previous studies (Penpargkul et al. 1981; Lopaschuk et al. 1983; Ganguly et al. 1983) have attempted to show that the cardiac SR dysfunction is paralleled by depression in hemodynamic parameters related to cardiac function in
STZ injected rats. It was, therefore, decided to study SR Ca\textsuperscript{2+} uptake in these spontaneously diabetic animals using both BB non-diabetic littermates and Wistar rats as controls. Wistar rats were included as controls since the pathological characteristics of BB rats are varied and, in part, time-dependent (Chappel and Chappel 1983); genetically susceptible rats may be "potential" diabetics without diagnosis during the follow up. This is of considerable importance to recognize because "littermate controls" without overt diabetes may be abnormal and may not be appropriate "controls" for many types of study (Nakhooda et al. 1978; Marliss et al. 1982).

Hearts from BB low dose (LD), high dose (HD) and non-diabetic littermates exhibited a depression in Ca\textsuperscript{2+} uptake when compared with age-matched Wistar controls. However, when SR Ca\textsuperscript{2+} uptake of the LD, HD and non-diabetic BB littermates were compared to each other, no significant differences were found between the 3 groups (Figure 23). Also, both LD and HD BB diabetic groups and their non-diabetic littermates showed hyperlipidemia as compared to Wistar controls (Figure 22). In fact, quantitative alterations of lipid and lipoprotein composition in BB rats, when compared to the Wistar rats, have also been documented by other workers (Patel et al. 1984). The present results on SR Ca\textsuperscript{2+} uptake and lipid metabolism, together with other strain related differences (Wright et al. 1983), suggest that the BB rat is not simply a "normal" Wistar rat that develops beta-cell necrosis, glycosuria, and ketosis; it also possesses other abnormalities induced by the process of inbreeding and the diabetic trait is only one component of an overall BB syndrome (Wright et al. 1983; Patel et al. 1984).

It is possible that these strain-related alterations in lipid metabolism may result in an altered lipid environment which contributes to the depressed SR function that we see in the BB rat heart. If tissue lipid levels rise, they may modify membrane integrity by disrupting important interactions that exist between the hydrophobic region of membrane protein and the lipid bilayer (Katz and Messineo 1981). This is important
because the presence of a lipid milieu is essential for maintaining the function of SR ATPase. Indeed, in vitro modulation of membrane lipid composition results in alterations in enzyme activity in skeletal SR which has a similar Ca\textsuperscript{2+}-uptake mechanism to that of cardiac SR (Madden et al. 1979). In addition, cardiac calcium uptake is markedly diminished in Yorkshire swine fed an atherogenic diet. In these animals, the amount of cholesterol per mg SR protein and the cholesterol:phospholipid ratio are elevated (Jacobson et al. 1985). In the present experiments also, the results indicate that the BB rats generally had higher lipid levels and showed an attenuated SR Ca\textsuperscript{2+}-uptake activity.

Furthermore, although ATP-dependent Ca\textsuperscript{2+}-uptake was significantly depressed in cardiac SR isolated from BB rats, a generalized depression in heart hemodynamic functions (LVDP, +/− dP/dt) did not result (Figure 24). This observation of a markedly depressed SR Ca\textsuperscript{2+}-uptake with normal contractility is not unique. Previous observations have shown that treatment of the diabetic rat with methyl palmoxirate (Tahiliani and McNeill 1985) or carnitine (Lopaschuk et al. 1983) could return SR function to normal without any effect on the cardiac depression. In addition, decreased Ca\textsuperscript{2+}-uptake by the SR with preserved, or even increased, contractility have been reported during experimentally induced uremia in rats (Penpargkul et al. 1976). One interpretation of the data is that in these BB rats, the in vitro cardiac SR preparation is not truly representative of cardiac SR function in vivo or that the SR does not have to be maximally transporting calcium in vivo to adequately maintain cytosolic calcium. The results also suggest that the SR may not be the limiting factor in controlling contractility in BB hearts since contractility is controlled by other mechanisms as well (Caroni and Carafoli 1981).

The reason as to why the LD BB diabetic rats do not develop cardiac dysfunction is unknown at this stage. However, the initial results were substantiated by Paulson and co-workers (1988). They showed that 8 weeks of diabetes in
BB/Worcester rats does not cause significant alterations in cardiac contractile function in perfused hearts \textit{in vitro}. One explanation of these results has been suggested by Malhotra et al. (1985). Using BB/Worcester rats, they reported that calcium-stimulated myosin ATPase activity was significantly depressed only after 4 and 7 months of diabetes and that the differences between hearts from control and diabetic animals increased with the duration of diabetes. They also suggested that although the biochemical findings in hearts from BB rats are qualitatively similar to previous findings in hearts from STZ diabetic rats, they differ in the time of appearance of abnormalities relative to the onset of diabetes. It is thus possible that because the spontaneously diabetic rats, and not the STZ-diabetic rats, require daily administration of insulin for survival, insulin treatment may have delayed the onset of cardiac abnormalities (Malhotra et al. 1985). Thus, it could be argued that the time of our study, and that of Paulson's (1988), was too short. It would be interesting to see what happens to cardiac function if the duration of the diabetic state was increased. It should also be pointed out that although the low dose insulin used in this study was about 1/3 of the amount necessary to optimally control hyperglycemia and glycosuria in BB (Gotfredsen et al. 1985) or STZ-induced (Rasch 1979) diabetic animals, the rats in this study were only moderately diabetic. Hence, experiments with more severely diabetic rats were designed and subsequently carried out. It should be noted however that these observations, taken together with the results of WKY and hydralazine studies, could also imply that factors other than hyperglycemia may be contributing to the depression in heart function noted during diabetes. Rubinstein et al. (1984) have reported that STZ-diabetic rats treated with insulin demonstrated correction of both isolated papillary muscles mechanics and actomyosin measurements, despite the fact that they had persistent hyperglycemia. That hypoinsulinemia, and not hyperglycemia, is the more important factor in diabetic cardiomyopathy, is also suggested by the present results.
In a subsequent experiment, BB diabetic rats were treated with an even lower insulin dose (3.5 U.Kg^{-1}.day^{-1}) such that they showed persistent hyperglycemia (Table 6). Upon sacrifice, plasma triglycerides were found to be drastically elevated, confirming the severity of the diabetic state (Figure 25). The magnitude of hyperlipidemia was one major difference between this and the previous study and it closely resembled the hyperlipidemia seen in STZ-induced diabetic rats (Rodrigues and McNeill 1986). For plasma cholesterol, there were strain-related but no diabetes-related differences as seen in the earlier study (Figure 25).

Definite differences in plasma insulin levels between BB non-diabetic and Wistar control rats were also apparent with no changes in plasma glucose (Table 6). Similar results by others have also indicated that although pancreatic immunoreactive insulin concentration in some BB non-diabetic rats is reduced to levels well below those in other experimental animals, these rats do not become overtly diabetic (Seemayer et al. 1983). These observations underline the extent of beta-cell loss required before overt diabetes occurs. It also confirms the earlier findings that littermate controls without diabetes may be abnormal and are not appropriate controls for many types of study (Marliss et al. 1982). The only justification for such a comparison in this study is that isolated hearts from BB non-diabetic littermates have previously been shown to have cardiac function similar to that seen in Wistar control rats.

Malhotra et al. (1985) examined contractile protein function in BB diabetic rats and found depressed Ca^{2+}-stimulated myosin and actomyosin ATPase activities. This depression would be expected to have an important impact on contractile performance of the heart. In this study, hearts from BB diabetic rats also exhibited a depression in myosin Ca^{2+}-ATPase activity which could explain the observed cardiac dysfunction (Table 6). Since the effects of thyroid hormones on cardiac contractile proteins are significant in a variety of conditions, some reports have suggested that alteration in the level of thyroid hormones may also affect contractile proteins and hence cardiac
function in STZ-induced diabetic rats (Dillman 1980). However, this study (Table 6) and others (Sternathal et al. 1981; Wright et al. 1983) have shown that thyroid hormone levels in BB diabetic animals are similar to those of non-diabetic littermates. This supports the view that hypothyroidism may not mediate the alterations in cardiac contractile proteins or cardiac function in this model of diabetes (Malhotra et al. 1985).

The observed cardiac dysfunction (Figure 26) could also be secondary to alterations in lipid metabolism, as evidenced by a rise in plasma triglycerides (Figure 25). Plasma fatty acids and heart triglyceride levels have also been reported to be elevated in BB diabetic animals (Nakhooda et al. 1978). High plasma lipid concentrations have a direct detrimental effect on myocardial performance (Opie 1970). Lipids also accumulate within cells where they may (a) contribute to an enhanced myocardial stiffness (Regan et al. 1974), (b) modify the structure of sarcolemmal and other subcellular membranes by their detergent-like effects (Katz and Messineo 1981), and (c) interfere with various cellular functions by specifically inhibiting critical enzyme systems (Wood et al. 1977; Lopaschuk et al. 1983).

In conclusion, these results suggest that the degree of insulin deficiency and the severity of the diabetic state are important determining factors for the development of diabetic cardiomyopathy and, to our knowledge, represent the first demonstration of a cardiomyopathy in the BB rat. The study also demonstrates that abnormal cardiac biochemistry and function in the spontaneously diabetic BB rat are similar to changes previously demonstrated after STZ or alloxan-induced diabetes. This indicates the suitability of the BB diabetic rat as a good model to study cardiac disease which is known to occur during IDDM in man.

One possible approach to improve cardiac function in diabetic rats is the use of insulin (Lopaschuk et al. 1983; Tahiliani et al. 1983). However, widespread, complex metabolic alterations are observed in both the untreated and insulin-treated diabetic rats. This prevents the drawing of firm conclusions regarding the mechanism of action
of insulin in improving cardiac function. In addition, since the previous studies indicated that hyperlipidemia, and not hyperglycemia, was important in altering cardiac functions in diabetic rats, a more specific means of altering lipid levels was sought. In this regard, a number of observations suggested that carnitine may be beneficial to the diabetic rat.

The total intracellular cardiac carnitine concentration in normal rats is approximately 20-40 fold higher than total plasma carnitine concentration implying that an active transport process exists for the transport of exogenous carnitine against a large concentration gradient. Indeed, although carnitine transport in tissue has been studied in a variety of systems, all have been shown to have a common saturable component (Vary and Neely 1982; Hamilton et al. 1983). In the heart, this component is optimally active at physiological plasma carnitine concentrations of 40-80 umole (Vary and Neely 1982). Thus, increasing the concentration of carnitine in the medium used to perfuse isolated rat hearts can result in a linear increase in the rate of carnitine transport into the myocardium within the range mentioned. Once the myocardial pool is filled, it is difficult to raise the amount of carnitine above a certain level (Vary and Neely 1983). These findings suggest that alterations in the levels of serum carnitine may result in an altered rate of myocardial carnitine uptake.

The literature records a great disparity of results from different investigators, as to the carnitine concentrations in plasma and heart during diabetes. For example, plasma total carnitine concentrations in diabetic animals have been observed to be either increased (Snoswell and McIntosh 1974) or decreased (Fogle and Bieber 1979). Similarly, hearts of diabetic animals have been reported to have increased total carnitine (Liu and Spitzer 1978), unchanged total carnitine (Person and Tubbs 1967) or decreased total carnitine (Vary and Neely 1982; Paulson et al. 1984). Some of these differences observed in the literature are due to differences in species, the severity of diabetes, time after induction of diabetes and the individual tissues examined.
In recent years, it has become clear that alloxan- or STZ-diabetic rats have reduced myocardial carnitine levels (Vary and Neely 1982; Paulson et al. 1984; Pieper et al. 1984; Pieper and Murray 1987; Rodrigues et al. 1988). Various reasons have been proposed for this finding. Vary and Neely (1982) showed that the decrease in cardiac carnitine in alloxan-diabetic rats was due to a decrease in plasma carnitine concentration (which in their animals fell below the $K_m$ for transport) rather than a defect in the myocardial transport of carnitine (which was unaltered). It should be pointed out that although generally accepted, this mechanism does not always hold true. For example, Brooks et al. (1985) showed that diabetic animals fed a diet rich in carnitine had higher plasma carnitine, and lower cardiac carnitine levels, than diabetic animals fed a diet without carnitine. The authors suggested that: (a) tissues can regulate the concentration and distribution of carnitine by active mechanisms beyond merely responding to plasma carnitine (Bohmer and Molstad 1980), and (b) alterations could occur in membrane-bound cardiac carnitine-binding proteins [which may transport carnitine into heart cells (Cantrell and Borum 1982)] resulting in the reduction of carnitine transport into diabetic hearts.

How do plasma carnitine levels in diabetic rats decrease? Various mechanisms have been postulated to explain this. These include (a) excessive degradation of carnitine; (b) alteration in cellular mechanism for carnitine transport affecting uptake and/or release of carnitine from tissue; (c) a defect in carnitine biosynthesis/secretion by the liver; (d) abnormal renal handling of carnitine, and (e) defective gastro-intestinal absorption of dietary carnitine (Rebouche and Paulson 1986; Bieber 1988). Carnitine deficiency could also occur secondary to increased excretion of carnitine. Free carnitine in plasma makes up a large percentage of the total carnitine concentration. During diabetes, carnitine deficiency and a depression of mitochondrial beta-oxidation rates leads to an increase of acyl carnitine at the expense of free carnitine. Acyl carnitine leaves the cells and is excreted in the urine, as it is less efficiently reabsorbed.
than free carnitine in the kidney's tubular reabsorption process (Engel et al. 1981; Bahl and Bressler 1987). The preferential reuptake of free carnitine by the kidneys results in clearance of the acyl carnitine that might otherwise be potentially toxic (each tissue, and the body as a whole, keeps a low acyl CoA to free CoA ratio by excretion of acyl carnitine). However, this leads to a deficiency of free carnitine in the plasma. In fact, Brooks et al. (1985) have shown that induction of diabetes produced a marked increase in urinary carnitine whereas insulin treatment rapidly reduced urinary carnitine concentration.

What are the consequences of a decrease in plasma and, hence, myocardial content of carnitine in the diabetic heart? Generally, the heart maintains a large reserve (about five fold) of carnitine relative to the amount necessary for long-chain fatty acid oxidation (Wittel and Bressler 1964). Hence, the absolute level of carnitine required to maintain normal cardiac function is unknown. Increasing evidence however indicates that carnitine deficiency may play an important role in the pathogenesis of cardiomyopathy. For instance, a cardiomyopathic hamster animal model that has low cardiac carnitine concentrations has been described (York et al. 1983; Yamashita et al. 1985). Rats (Reibel et al. 1983) and rabbits (Reves and Cameron 1979) subjected to surgically induced pressure overload develop cardiac hypertrophy with reduced myocardial carnitine content. In the diphtheria-infected guinea pig, Wittels and Bressler (1964) showed that this toxin produced a depletion of myocardial carnitine which resulted in cardiomyopathy. Myocardial carnitine stores also significantly decrease with age (Abu-Erreish et al. 1977) and diabetes (Vary and Neely 1982; Paulson et al. 1984) with subsequent effects on contractile function. A number of observations have also demonstrated that the cardiac defects associated with decreased carnitine stores can be alleviated by administration of L-carnitine. For example, long-term L-carnitine treatment of cardiomyopathic hamsters (Yamashita et al. 1985; Whitmer 1987) and aged rats (Lucreziotti et al. 1983) resulted in restoration of
myocardial carnitine stores and significantly increased mechanical performance. Carnitine has been found to protect the myocardium against diphtheria-induced myocardial toxicity [in animals (Bressler and Wittles 1965) and humans (Ramos et al. 1984)] and myocardial ischemic changes [in clinical (Thomsen et al. 1979) or experimental settings (Liedtke and Nellis 1979; Nagao et al. 1987)]. A cardiomyopathy induced by adriamycin, an anti-cancer drug, can be prevented by L-carnitine supplementation (McFalls et al. 1986). In the diabetic heart also, both indirect and direct methods of increasing carnitine stores have beneficial effects. For example, carnitine contains three methyl groups initially derived from methionine or choline and thus a low availability of methionine or choline would result in a significant decrease in carnitine levels. Both choline and methionine levels are decreased in diabetic rats and treatment of diabetic rats with these two agents has been shown to improve cardiac function (Heyliger et al. 1986). Daily administration of verapamil to hamsters with hypertrophic cardiomyopathy also significantly increased total myocardial carnitine stores (Yamashita et al. 1985). Recently Afzal et al. (1988) showed that verapamil treatment of chronically diabetic rats was capable of preventing diabetes-induced myocardial changes. On a more direct basis, carnitine administration to acutely diabetic rats had beneficial effects on the heart. Perfusion of diabetic rat hearts with physiological concentrations of palmitate caused a time- and concentration-dependent reduction in myocardial ATP content and an increase in long chain acyl CoA in vivo. Similar results were also obtained by perfusion with palmitate concentrations simulating elevated serum fatty acid levels (Pieper et al. 1984; Pieper and Murray 1987). Addition of carnitine to the perfusion medium or its administration before death in vivo attenuated or prevented both the loss of ATP and increase in long-chain acyl CoA (Pieper et al. 1984; Pieper and Murray 1987). These hearts were also less vulnerable to ischemia leading to an improvement in contractile function (Paulson et al. 1984).
On the basis of these reports, it was decided to administer carnitine to rats from the onset of diabetes to see if this intervention could replenish total myocardial carnitine levels and possibly prevent the depression in heart function after chronic diabetes. In previous studies, DL-carnitine had been employed. The results indicated that oral DL-carnitine treatment did not prevent the onset of heart dysfunction in diabetic rats (Lopaschuk et al. 1983). Subsequently, it was shown that the L-isomer of carnitine, not the D-isomer, is the physiologically active form. D-carnitine, in fact, inhibits carnitine esterification and can produce a decrease in myocardial carnitine content (Paulson and Shug 1981). Also, the carnitine transport system possesses about a 25-fold greater affinity for the L-isomer than the D-isomer (Vary and Neely 1982).

Injection of L-isomer of carnitine i.p. restored the myocardial free-carnitine fraction in diabetic rats to levels comparable to control values (Table 8). Associated with this increase in myocardial carnitine content was a prevention of the onset of heart dysfunction in chronically diabetic rats (Figures 29-31). The reversibility of diabetes-induced myocardial alteration by L-carnitine administration was studied next. Injection of L-carnitine for two weeks to rats diabetic for six weeks partially reversed the adverse effects of chronic diabetes on heart function (Figure 35). The treatment period was probably too short to fully restore cardiac function and it would be interesting to study the effect of prolonging the duration of L-carnitine treatment. Although these experiments and others (Paulson et al. 1984; Rodrigues et al. 1988) showed that high doses of L-carnitine were effective in alleviating some of the problems associated with diabetes, experiments were not done to assess the optimal doses required. Therefore, it was decided to study the effects of low dose L-carnitine on cardiac function. Exogenous low-dose L-carnitine treatment for six weeks prevented the onset of heart dysfunction of chronically diabetic rats (Figure 36). These results indicated that L-carnitine, either in acute or chronic studies or in low or high doses has a protective effect against myocardial damage seen during diabetes.
The precise mechanism of this improvement in cardiac function in diabetic animals is unknown, but it could be due to a number of reasons. For example, the increased incidence of cardiac dysfunction in diabetic patients or drug-induced animal models could be secondary to alterations in lipid metabolism which probably modify the structure of cardiac plasma and subcellular membranes. Diabetes, which accelerates the rate of lipid metabolism in the heart, results in an elevation within the cytosol of a number of lipids and/or intermediates involved in lipid metabolism, including acyl CoA and long-chain acyl carnitines (Lopaschuk et al. 1983; Paulson et al. 1984; Pieper et al. 1984; Pieper and Murray 1987). If tissue levels of these intermediates rise markedly (as seen during carnitine deficient states), deleterious effects on cardiac sarcolemma and subcellular membrane structure or function can occur (Vaartjes et al. 1973; Wood et al. 1977; Katz and Messineo 1981; Lopaschuk et al. 1983). They also interact directly with Ca\(^{2+}\) channels and may therefore be considered as endogenous modulators of channel function (Spedding and Mir 1987) and may account for the deleterious Ca\(^{2+}\) overload following diabetes (Afzal et al. 1988). These lipid intermediates also cause a depression in ATP production (Pieper et al. 1984; Pieper and Murray 1987). Thus, the improvement in cardiac function in the diabetic animals in the present study may be due to a decrease in the accumulation of fatty acid intermediates such as acyl CoA and long-chain acyl carnitines, which have been shown to decrease after L-carnitine administration in the diabetic rat (Paulson et al. 1984; Pieper et al. 1984; Pieper and Murray 1987).

Another possible mechanism for the improvement in cardiac function after L-carnitine treatment may be its ability to lower blood lipids. In all three studies, the effect of L-carnitine in lowering blood lipids in diabetic rats was quite pronounced (Figure 32, Table 9 and 11). A hypolipidemic effect of carnitine has also been found in patients on hemodialysis (Casciani et al. 1980; Vacha et al. 1983), new-born infants (Schmidt-Sommerfeld et al. 1960), patients with Type IV hyperlipoproteinemia
(Maebashi et al. 1978; Pola et al. 1980), diabetes (Bakaert and Deltour 1960) and uremia (Guarnieri et al. 1980) and in experimental hyperlipidemic conditions where L-carnitine administration is capable of reducing hyperlipidemia after oral oil bolus (Maccari et al. 1980) or fat diet feeding (Maccari et al. 1985; Mondola et al. 1988). The reason why serum lipids are not elevated in these L-carnitine-treated diabetic rats despite insulin deficiency is unknown. However, because carnitine is a prerequisite for normal transport of fatty acids into the mitochondria for their oxidation, the positive effects of L-carnitine on certain pathological or experimental hyperlipidemic conditions are generally explained by its ability to stimulate mitochondrial beta-oxidation at the expense of extramitochondrial triglyceride synthesis. Beta-oxidation causes free fatty acid removal from hepatic triglyceride synthesis resulting in the reduction of both free fatty acids and triglycerides in the serum. Indeed, studies have shown that fatty acid oxidation is stimulated by the addition of carnitine to heart homogenates or isolated mitochondria (Fritz and Yui 1963). There was also a positive correlation between plasma beta-hydroxybutyrate and plasma total carnitine levels which supported a carnitine-related enhancement of mitochondrial fatty acid oxidation (Schmidt-Sommerfeld 1982). However, the addition of carnitine to intact tissues does not always enhance fatty acid uptake and oxidation (Liedtke et al. 1981; Brady et al. 1986). These results suggest that other mechanisms can also be postulated for the hypolipidemic effect of L-carnitine.

In obese rats, for example, the mechanism of action of carnitine in decreasing triglycerides appear to be via decreased triglyceride synthesis and/or secretion by the liver (Brady et al. 1986). This was based on a significant increase in hepatic lipid levels and an inhibition of hepatic triglyceride secretion. L-carnitine did not increase hepatic mitochondrial oxidative capacity in these rats (Brady et al. 1986).

Liedtke and Nellis (1979) have also indicated that acute L-carnitine administration beneficially affects cardiac function by decreasing, rather than
increasing, the use of fatty acids. They found that carnitine administration to hearts perfused with high levels of fatty acid (a situation similar to that seen in diabetes) was able to prevent the intracellular accumulation of fatty acids. Rodis et al. (1970) using isolated perfused rat hearts reported similar observations. They demonstrated that carnitine in the perfusate depressed exogenous palmitate uptake and slightly decreased fatty acid oxidation in aerobic muscle. Thus, carnitine, by decreasing the uptake and oxidation of fatty acids, may facilitate the removal of these acyl compounds by eliminating them in the urine in the form of acyl carnitines. In fact, a recent review by Stumpf et al. (1985) focussed on this particular role of carnitine and suggested it to be the most important clinical role of carnitine established as yet. These authors defined carnitine deficiency as follows: "carnitine deficiency exists when there is insufficient carnitine to buffer toxic acyl compounds. In eliminating such acyl compounds, a tissue may have a net efflux of carnitine and a syndrome of carnitine deficiency may result".

If carnitine indeed decreases fatty acid uptake and oxidation in the heart, it could improve the use of other substrates (which in turn help maintain high energy phosphate stores). An excessive oxidation of long-chain fatty acids generated by hydrolysis of endogenous triglycerides or taken up from the plasma is at least partly responsible for the depression of glucose oxidation and insulin resistance in the diabetic heart according to the hypothesis of Randle et al. 1963. For example, it is well known that in the diabetic heart, glucose transport is less sensitive to stimulation by insulin (Randle et al. 1966), whereas insulin accelerates glucose uptake and metabolism not only in the Langendorff heart (Randle et al. 1966) but also under a physiological workload (Sudgen and Smith 1982). Increased fatty acid oxidation leads to inhibition of glucose metabolism at several sites such as pyruvate dehydrogenase (Kerbey et al. 1976) and phosphofructokinase (Parssoneau and Lowry 1963). This is because excessive fatty acid oxidation increases acetyl CoA:CoA, ATP:ADP and NADH:NAD ratios which activate pyruvate dehydrogenase kinase resulting in inactivation of pyruvate
dehydrogenase. The citrate thus formed may inhibit phosphofructokinase, thereby inhibiting the glucose utilization (Randle 1985). In fact agents (2-tetradecylglycidic acid; 2[5(4-chlorophenyl) pentyl] oxirane-2-carboxylic acid) that strongly inhibit oxidation of long-chain fatty acids correct derangements such as fatty acid induced inhibition of glucose oxidation, and are effective hypoglycemic agents in fasted or diabetic animals. With these agents, the hypoglycemia is a consequence of inhibited gluconeogenesis and increased glucose utilization (Tutwiler et al. 1979; Seitelberger et al. 1984) probably by reactivation of the pyruvate dehydrogenase complex. Opie (1979) has shown that carnitine repletion in rat hearts accelerated the rate of glycolysis by increasing the activity of pyruvate dehydrogenase and enhancing the utilization of glucose oxidative metabolism. Carnitine may therefore affect energy metabolism by a mechanism opposite to what one would expect, i.e., decreasing rather than increasing fatty acid oxidation. If this were true, then the better preservation of function in diabetic hearts may partly result from this concomitant stimulation of glucose oxidation (Neely and Morgan 1974).

As shown in these studies (Figure 28), the effects of L-carnitine on blood glucose are consistent with the results obtained by Paulson et al. (1984). They reported that acute L-carnitine treatment of diabetic rats for 2 weeks significantly reduced serum glucose. However, the reduction in blood glucose was most pronounced in the prevention study and probably is a result of a longer treatment period (Figure 28). In this regard, Pieper and Murray (1987) showed that L-carnitine injection for two days did not reduce blood glucose in their diabetic animals. The reduction in blood glucose in the present studies was independent of the levels of insulin, which remained depressed in the diabetic animals. As discussed earlier, it is possible that reduction of glucose levels is an indirect consequence of removal of lipid or lipid intermediates or a reduced food intake. This reduction of blood glucose could explain the absence of
glycosuria and polydipsia seen throughout the prevention study in these diabetic animals.

Whether the lipid-lowering effect of carnitine in the present study was due to an increased fatty acid oxidation, decreased fatty acid uptake and hence excretion in the urine, or, alternatively, due to decreased hepatic synthesis or secretion of triglycerides is not clear. However, the effect of L-carnitine on adipose tissue lipolysis was investigated. Triglyceride lipase is the rate determining enzyme in adipose tissue lipolysis. It is an insulin-inhibited, adenylate cyclase-dependent enzyme. Under conditions of insulin deficiency, triglyceride lipase acts in an uninhibited manner and causes a marked increase in adipose tissue lipolysis. This results in the release of free fatty acids as well as glycerol in large quantities from adipose tissue (Kreisberg 1966). L-carnitine treatment for 2 weeks to rats previously diabetic for 6 weeks reduced the basal lipolysis rate, which could be another mechanism by which L-carnitine lowers lipid levels (Table 9). Since it has been postulated that an inhibition of lipolysis and long-chain fatty acid oxidation might be a useful approach to the treatment of diabetes mellitus (Chase and Tubbs 1972; Tutwiler et al. 1979), this could be an important property of carnitine. This is because high lipid concentrations have a direct negative effect on myocardial performance, probably by limiting the amount of calcium available to the myofibrils (Henderson et al. 1970). Opie (1970) reported that perfusion of isolated rat hearts with a medium containing 2.8 mM octanoate produces atrioventricular conduction disturbances. Willebrands et al. (1973) perfused isolated rat hearts with a balanced salt solution containing human albumin (0.66 mM) and oleic acid and found that amplitude of contraction decreased more rapidly with time in experiments with oleic acid than in those without. Since diabetic hearts have depleted carnitine levels, they are also unable to handle higher circulating levels of lipid substrates. This is presumably responsible for the intracellular accumulation of lipid intermediates which then not only participate in extramitochondrial reactions such as
triglyceride synthesis (Denton and Randle 1967) but also inhibit triglyceride lipase activity (Severson and Hurley 1982). The resulting effect is an increased tissue level of triglycerides in the diabetic heart (Denton and Randle 1967; Heyliger et al. 1986), one of the factors contributing to enhanced myocardial stiffness (Regan et al. 1974). Thus, in keeping with the previous results, the lack of cardiac dysfunction in L-carnitine-treated diabetic rats may partly be explained by the fact that these animals have a normal lipid metabolism.

The question also arises as to whether this salutary effect of L-carnitine on myocardial performance was independent of any direct cardiac alteration. In patients with moderately impaired left ventricular function, intravenously administered L-carnitine exerted a positive inotropic effect (Thomsen et al. 1979). The effects of L-carnitine on cardiac hemodynamics were also evaluated in normal dogs (Brooks et al. 1977; Suzuki et al. 1981) and were as follows: heart rate decreased, aortic and left ventricular pressure increased, peak left ventricular +dP/dt increased, coronary blood flow increased (this being the most pronounced effect) and coronary vascular resistance decreased. The onset of action by carnitine was fairly rapid and its effect transient, promptly disappearing as the infusion was stopped. The mechanism of this vasodilation and positive inotropic effect could not be explained as they were not blocked by propranolol or by atropine and were still present in reserpinized, catecholamine-depleted animals (Brooks et al. 1977). The results of the present study indicate that L-carnitine had no direct effect on mechanical function of the isolated working rat heart (Figure 37). This result is similar to the experiments of Pieper et al. (1984) but contrary to those of others (Brooks et al. 1977; Suzuki et al. 1981) who showed positive inotropic responses with \textit{in vivo} dog preparations. The cardiac changes observed in this study therefore appear to be independent of any direct pharmacological effects on the myocardium and may be due to an improvement in fatty acid metabolism which is the primary property of carnitine.
In any pharmacological study, both the dose and route of administration are important factors in establishing an effect. In earlier studies, oral doses of DL-carnitine were employed and the results indicated that carnitine treatment did not prevent the onset of heart dysfunction in diabetic rats (Lopaschuk et al. 1983). It is not known whether this lack of response was due to the use of DL-carnitine (instead of the L-isomer) or the oral route of administration (instead of the i.p. route). Thus, experiments were designed to evaluate the effects of oral L-carnitine treatment on cardiac function in chronically diabetic rats using the isolated perfused heart. Results with L-carnitine given orally (pilot study) indicated that one (Table 12, Figure 39) and three (Table 13, Figure 40) weeks after L-carnitine treatment, plasma total and free carnitine levels were increased, acyl carnitine levels were decreased, and there were beneficial effects on lipid metabolism. However, chronic treatment (6 weeks) with L-carnitine (given orally) was ineffective in treating these animals. Plasma total and free carnitine levels remained low, plasma lipids and acyl carnitines were still elevated, myocardial carnitine stores did not increase and cardiac function remained depressed (Table 14, Figures 41 and 42). Other authors also noted that oral and injected doses of L-carnitine show varying effects. York and colleagues (1983) reported that oral L-carnitine treatment via the drinking water did not increase cardiac concentrations of carnitine in cardiomyopathic Bio 14.6 hamsters, whereas Yamashita et al. (1985) reported that chronic i.p. administration of L-carnitine significantly increased total myocardial carnitine stores in these same animals. Similarly, when oral doses of L-carnitine were given to obese Zucker rats (Brady et al. 1986), plasma levels never reached an effective therapeutic value as compared to i.p. doses. The authors suggested that the absorbed oral dose apparently did not increase plasma levels to the same extent as i.p. doses and that this most likely represents poor oral absorption from the gastrointestinal tract and subsequent metabolism of carnitine by colon bacteria. Indeed, studies in humans (Rebouche and Engel 1984), dogs (Rebouche and Engel
and rats (Brooks and McIntosh 1975; Cedarblad and Lindstedt 1976) have suggested that a significant amount of dietary carnitine is degraded in the gastrointestinal tract. Conclusive evidence demonstrating the part of indigenous flora of rat gastrointestinal tract in this process was recently reported (Rebouche et al. 1984).

The discrepancy between oral and i.p. treatment could also be ascribed to other causes. For instance, carnitine at elevated concentrations down-regulates its own transport across the renal brush border membrane in vitro (Rebouche and Mack 1984). It is possible that such a mechanism may also be operational in the gut of diabetic rats given L-carnitine orally. Whatever the mechanism, our results (like others) indicate that L-carnitine given orally is ineffective in treating diabetic rats. Thus the lack of a beneficial response to carnitine therapy in some patients and animals may simply be due to the route of administration.

In summary, L-carnitine treatment of rats with experimentally induced diabetes improved cardiac function. It is possible that carnitine deficiency contributes to muscle dysfunction because of reduced energy production. This could promote structural disruption of the myocardium at a cellular level through deposition of lipids in the myofibrils and through structural and functional subcellular membrane alterations which eventually lead to poor contractile performance. Pretreatment with L-carnitine could possibly help maintain tissue ATP levels and reduce the accumulation of lipid metabolic intermediates, resulting in improved use of other substrates like glucose.

Since aberrant carnitine metabolism has such severe consequences on the myocardium, an understanding of the biochemical mechanisms controlling the biosynthesis, inter-organ transport and excretion of carnitine during experimental diabetes is needed. In humans, procedures for the accurate assessment of carnitine and lipid status need to be improved and applied to a larger population of patients than studied in the past. It is imperative to good patient care that those experiencing carnitine deficiency be identified and treated. Although the therapeutic usefulness of
L-carnitine in human remains to be elucidated, the supplementation of patients with L-carnitine (or other lipid lowering drugs) could provide a unique metabolic approach to the therapy of diabetes and holds promise as an adjuvant to traditional therapies. The results also indicate that lowering of lipid levels could be an attractive approach for the normalization of glucose metabolism and insulin insensitivity during diabetes.
1. Induction of diabetes in rats with streptozotocin causes profound cardiovascular changes which result in depressed performance of isolated working heart.

2. In close parallel to the depression of heart function, various biochemical changes occur during diabetes. These include hypoinsulinemia, hyperglycemia, hyperlipidemia and elevated myocardial lipid levels.

3. Of these biochemical changes, hyperlipidemia appears to be a major contributing factor to the development of cardiac dysfunction during diabetes. For example, experimentally induced diabetes did not alter cardiac function or lipid metabolism in WKY rats but severely affected these indices in Wistar rats. Since the essentially unaffected WKY diabetic rats and the severely affected Wistar diabetic rats received the same STZ dose and attained the same degree of hyperglycemia, it is not likely that these effects are due to a difference in severity of the diabetic state in the two groups.

4. Similarly, hydralazine treatment of experimentally induced diabetic rats improved cardiac function and lowered lipid levels. The lipid lowering effect of hydralazine, apparently, does not occur at the site of adipose tissue free fatty acid mobilization. Irrespective of the mechanism(s) involved in lowering blood lipids, the results are important. This is because first line drugs currently used for the treatment of the hypertensive-diabetic patients (thiazide diuretics and propranolol) may themselves cause elevation of
triglycerides and cholesterol. Since it is important to treat diabetic hypertension with drugs that neither cause nor worsen hyperlipidemia, hydralazine could prove to be an important agent, especially if its lipid lowering effect persists when the drug is titrated to achieve an optimal antihypertensive response.

5. Metabolic alterations and cardiac functional changes in the spontaneously diabetic BB rats are similar to changes previously demonstrated after STZ- or alloxan-induced diabetes. This indicates the suitability of the BB diabetic rat as a good model to study cardiac disease which is known to occur during IDDM in man. However, in using the BB rat, it should be understood that this species is not simply a "normal" Wistar rat that develops beta-cell necrosis, glycosuria and ketosis; it also possesses other abnormalities induced by the process of inbreeding and the diabetic trait is only one component of an overall syndrome that the BB rats exhibit.

6. In the BB studies, the results also indicated that since persistent hyperglycemia does not appear to be sufficient to cause cardiac dysfunction, other factors must contribute to the depression in heart function noted during diabetes. In this regard, it was only when plasma triglycerides were drastically elevated in these rats that cardiac dysfunction was observed. In addition, since BB rats showed a depressed SR Ca^{2+}-uptake with normal cardiac function, it is possible that these events are not related in these animals.

7. Many of the lipid abnormalities and hence cardiac dysfunction seen during diabetes could be a result of carnitine deficiency. Indeed, L-carnitine
treatment of diabetic rats prevented and reversed cardiac dysfunction. These effects of L-carnitine were independent of any direct cardiac alteration but were probably due to its powerful hypolipidemic action. This property of L-carnitine could be a matter of considerable interest since the incidence of atherosclerosis is much greater in diabetic than in non-diabetic humans and is related to hyperlipidemia.

8. Since all the above studies suggest that aberrant lipid metabolism has severe consequences on the myocardium, one should focus not only at maintaining strict glucose control in diabetes, but also in controlling lipid levels.

9. The present work also suggests that possible pharmacological approaches (evidently, the use of hypolipidemic agents), in addition to the use of insulin, should be explored in search of an ultimate treatment of the serious, and frequently fatal, cardiovascular complications of chronic diabetes.

10. Although the various studies suggested that there is correlation between hyperlipidemia and cardiac dysfunction during diabetes, further investigation should be undertaken to fully confirm this observation.
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