EFFECTS OF MYO-INOSITOL AND, OR
TRIIODOTHYRONINE (T3) TREATMENT ON CARDIAC DYSFUNCTION
AND ELEVATED MYOCARDIAL LIPID LEVELS
IN STZ-DIABETIC RATS

by

Hong Xiang

B.Med., Beijing Medical University, P.R.C., 1984

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in
THE FACULTY OF GRADUATE STUDIES
Division of Pharmacology and Toxicology
of the Faculty of Pharmaceutical Sciences

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
September, 1987

© Xiang Hong, 1987
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Pharmaceutical Sciences

The University of British Columbia
1956 Main Mall
Vancouver, Canada
V6T 1Y3

Date Oct 13, 1987
ABSTRACT

A number of experimental studies have implied a link between diabetes-induced lipid accumulation in the myocardium and the development of cardiomyopathy. Since diabetics excrete large amounts of myo-inositol which is a lipotropic agent, this study was undertaken to investigate the effects of myo-inositol on the elevated myocardial lipid levels and the depressed cardiac performance of diabetic rats. Diabetes was induced in female Wistar rats (190-215 g) with streptozotocin (STZ) (55 mg/kg, i.v.). Three days after diabetes induction, myo-inositol was administered in the drinking water (2.5 g/kg/day) for an 8 week period. Untreated diabetics exhibited a loss of body weight, hyperglycemia, hypoinsulinemia and hypothyroidism. These effects were not altered after myo-inositol treatment. STZ-diabetes also produced a significant elevation of plasma and myocardial triacylglycerol, cholesterol and phospholipid. Myo-inositol treatment decreased these lipid levels. In addition, hearts from diabetic animals had a decreased ability to develop left ventricular developed pressure (LVDP) and both the rate of pressure rise (+dP/dt) and the rate of pressure decline (-dP/dt) were also reduced. Hearts from myo-inositol-treated diabetic animals showed a partial but definite improvement of cardiac function.

As diabetes-induced hypothyroidism was not altered after myo-inositol supplementation, a combination treatment of both myo-inositol (2.5 g/kg/day, p.o. daily) and T₃ (30 ug/kg/day, s.c. daily) was then undertaken to determine whether heart function of diabetic rats could be further improved. STZ-diabetic rats were characterized by a loss of body weight, hyperglycemia and hypoinsulinemia; none of which were altered by either T₃ or myo-inositol plus
$T_3$ treatment. $T_3$ treatment normalized the thyroid state of diabetic animals as shown by Tahiliani and McNeill (1984). However, plasma and myocardial triacylglycerol, cholesterol and phospholipid levels of diabetic rats either remained elevated or were further increased with $T_3$ or myo-inositol plus $T_3$ treatment. In addition, $T_3$ treatment alone did not prevent cardiac dysfunction in diabetic rats. There was, however, some improvement in heart function in the groups treated with both myo-inositol and $T_3$, but the improvement was not as pronounced as with myo-inositol treatment alone.

John H. McNeill, Ph.D.
Thesis Supervisor
TABLE OF CONTENTS

ABSTRACT ii

LIST OF TABLES vi

LIST OF FIGURES vii

LIST OF ABBREVIATIONS ix

ACKNOWLEDGEMENTS xi

DEDICATION xii

INTRODUCTION 1

I. Overview of diabetes mellitus 1

II. Diabetic cardiomyopathy 5

III. Diabetes and altered lipid metabolism in the heart and adipose tissue 8

IV. Treatments employed to correct lipid metabolism in diabetics 11

V. Myo-inositol and diabetes mellitus 13

VI. Thyroid hormones 17

SPECIFIC GOALS OF THE INVESTIGATION 20

MATERIALS AND METHODS 22

I. Materials 22

1. Animals 22

2. Chemicals 22

3. Assay kits 22
## II. Methods

1. **Induction of experimental diabetes**
2. **Treatment protocols**
   - (1) Myo-inositol preliminary study
   - (2) Myo-inositol treatment study
   - (3) Myo-inositol and triiodothyronine ($T_3$) treatment study
3. **Isolated working heart perfusion**
4. **Measurement of plasma triacylglycerol, cholesterol and phospholipid levels**
5. **Determination of myocardial triacylglycerol, cholesterol and phospholipid levels**
6. **Protein assay**
7. **Blood analysis**
8. **Statistical analysis**

### RESULTS

### DISCUSSION

### CONCLUSIONS

### REFERENCES
### LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Characteristics of Type I and Type II diabetes mellitus</td>
<td>3</td>
</tr>
<tr>
<td>II.</td>
<td>Effect of different concentrations of myo-inositol treatment on plasma glucose, plasma and myocardial lipids in 8 week diabetic rats</td>
<td>41</td>
</tr>
<tr>
<td>IIa.</td>
<td>Effect of different concentrations of myo-inositol treatment on fluid intake and estimated inositol intake of 8 week diabetic rats</td>
<td>42</td>
</tr>
<tr>
<td>III.</td>
<td>General features of experimental rats used for myo-inositol treatment study</td>
<td>43</td>
</tr>
<tr>
<td>IV.</td>
<td>Fluid and myo-inositol intake of experimental rats used for myo-inositol treatment study</td>
<td>44</td>
</tr>
<tr>
<td>V.</td>
<td>Thyroid status of experimental rats used for myo-inositol treatment study</td>
<td>45</td>
</tr>
<tr>
<td>VI.</td>
<td>General features of experimental rats used for myo-inositol and triiodothyronine treatment study</td>
<td>64</td>
</tr>
<tr>
<td>VII.</td>
<td>Fluid and myo-inositol intake of experimental rats used for myo-inositol and triiodothyronine treatment study</td>
<td>65</td>
</tr>
<tr>
<td>VIII.</td>
<td>Thyroid status of experimental rats used for myo-inositol and triiodothyronine treatment study</td>
<td>66</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>#</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Possible metabolic changes in diabetes</td>
<td>9</td>
</tr>
<tr>
<td>2.</td>
<td>Myo-Inositol metabolism cycle</td>
<td>14</td>
</tr>
<tr>
<td>3.</td>
<td>Effect of myo-inositol treatment on plasma triacylglycerols of control and STZ-diabetic rats</td>
<td>46</td>
</tr>
<tr>
<td>4.</td>
<td>Effects of myo-inositol treatment on plasma cholesterol of control and STZ-diabetic rats</td>
<td>48</td>
</tr>
<tr>
<td>5.</td>
<td>Effect of myo-inositol treatment on plasma phospholipid of control and STZ-diabetic rats</td>
<td>50</td>
</tr>
<tr>
<td>6.</td>
<td>Effect of myo-inositol treatment on myocardial triacylglycerols of control and STZ-diabetic rats</td>
<td>52</td>
</tr>
<tr>
<td>7.</td>
<td>Effect of myo-inositol treatment on myocardial cholesterol of control and STZ-diabetic rats</td>
<td>54</td>
</tr>
<tr>
<td>8.</td>
<td>Effect of myo-inositol treatment on myocardial phospholipid of control and STZ-diabetic rats</td>
<td>56</td>
</tr>
<tr>
<td>9.</td>
<td>Effect of diabetes and myo-inositol treatment on left ventricular developed pressure of isolated perfused working hearts at various filling pressures</td>
<td>58</td>
</tr>
<tr>
<td>10.</td>
<td>Effect of diabetes and myo-inositol treatment on positive dP/dt of isolated perfused working hearts at various filling pressures</td>
<td>60</td>
</tr>
<tr>
<td>11.</td>
<td>Effect of diabetes and myo-inositol treatment on negative dP/dt of isolated perfused working hearts at various filling pressures</td>
<td>62</td>
</tr>
</tbody>
</table>
12. Levels of triacylglycerols in plasma obtained from control and diabetic rats treated with myo-inositol and triiodothyronine ($T_3$)  
67
13. Levels of cholesterol in plasma obtained from control and diabetic rats treated with myo-inositol and $T_3$  
69
14. Levels of phospholipid in plasma obtained from control and diabetic rats treated with myo-inositol and $T_3$  
71
15. Measurement of myocardial triacylglycerol levels in control and diabetic rats administered myo-inositol and $T_3$  
73
16. Measurement of myocardial cholesterol levels in control and diabetic rats administered myo-inositol and $T_3$  
75
17. Measurement of myocardial phospholipid levels in control and diabetic rats administered myo-inositol and $T_3$  
77
18. Effect of 8 week myo-inositol and $T_3$ treatment in diabetic rats on diabetes-induced depression of LVDP  
79
19. Effect of 8 week myo-inositol and $T_3$ treatment in diabetic rats on diabetes-induced depression of $+dP/dt$  
81
20. Effect of 8 week myo-inositol and $T_3$ treatment in diabetic rats on diabetes-induced depression of $-dP/dt$  
83
LIST OF ABBREVIATIONS

MI  myo-inositol
STZ  streptozotocin
LVDP  left ventricular developed pressure
+dP/dt  rate of development of left ventricular pressure
-dP/dt  rate of decline of left ventricular pressure
T₃  3,5,3',5'triiodothyronine
T₄  3,5,3',5' tetraiodothyronine
CON  untreated controls
COI  myo-inositol-treated controls
COT  T₃-treated controls
CIT  myo-inositol-plus-T₃-treated controls
DIA  untreated diabetics
DII  myo-inositol-treated diabetics
DIT  T₃-treated diabetics
DIIT  myo-inositol-plus-T₃-treated diabetics
FFA  free fatty acid
LCAC  long-chain acylcarnitine
cmH₂O  centimeter of water

gram
kilo
kilogram
liter
milli
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>mL</td>
<td>millilitre</td>
</tr>
<tr>
<td>mmHg</td>
<td>millimeter of mercury</td>
</tr>
<tr>
<td>mm Hg/s</td>
<td>millimeter of mercury per second</td>
</tr>
<tr>
<td>ng</td>
<td>nanogram</td>
</tr>
<tr>
<td>nmoles</td>
<td>nanomole</td>
</tr>
<tr>
<td>U</td>
<td>unit</td>
</tr>
<tr>
<td>u</td>
<td>micro</td>
</tr>
<tr>
<td>SR</td>
<td>sarcoplasmic reticulum</td>
</tr>
<tr>
<td>IP₃</td>
<td>inositol-1,4,5-triphosphate</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENT

I wish to express my deepest gratitude to my supervisor Dr. John H. McNeill for moral support, knowledgeable direction and understanding which have enabled me to complete this work. I would also like to express my sincere thanks to Dr. Clayton Heyliger, Mr. Brian Rodrigues and Mr. Allan Prakash who have encouraged me and given me inspiration and invaluable support in this effort. The suggestions and scientific input from the members of the supervisory committee (Dr. J. Diamond, Dr. K. MacLeod, Dr. F.S. Abbott) are gratefully acknowledged.

A special thanks to Ms. Judy Wyne for typing this thesis. The financial support of the B.C. Heart Foundation is gratefully acknowledged.

Also my thanks to all my colleagues and associates for their enthusiasm and help.

Xiang Hong
DEDICATION

To my parents and brothers,
for their love
and all they have done.
INTRODUCTION

I. OVERVIEW OF DIABETES MELLITUS.

Diabetes mellitus is a very old disease of mankind. As far back as the Ebers papyrus (about 1500 B.C.), the phenomenon of polyuria was observed in Egypt and "honey urine" was noted by Sushrutha in India in 400 B.C. The first good clinical description of the disease was provided by Celsus (30 B.C. to A.D. 50), and the term "diabetes", which is Ionic Greek and means "to run through a siphon", was first introduced by another Roman physician, Aretaeus (A.D. 30 to A.D. 90). Descriptions of a disease (such as "malady of thirst") suggesting diabetes were made by Chinese and Japanese in these early centuries. An Arab physician Avicenna in about 1000 A.D. gave a very good description of diabetes as well as some of its complications such as gangrene (for reviews, see Best, 1960 and Marble, 1971). During the 17th century, the observations of Willis marked the beginning of the "diagnostic period" in the history of diabetes. He observed that the urine of diabetes was "wonderfully sweet" and Dobson (1775) stated that the sweetness was due to sugar. These led Cullen (1710-1790) to add "mellitus" to "diabetes" to separate diabetes mellitus from diabetes insipidus. Rollo (1797) started the period of diabetes treatment by prescribing a dietary regimen (low carbohydrate, high protein and fat). In 1869 Langerhans discovered the islet in pancreatic tissue which was named in his honour by Laguesse. The experimental period was initiated by Mering and Minkowski (1889) who produced diabetes in dogs by total pancreatectomy and first connected the diabetes with the pancreas. The greatest step forward came in 1921 when Banting
and Best succeeded in extracting from the islets of Langerhans a substance with hypoglycemic properties, which was later given the name "insulin". Subsequently oral hypoglycemic agents were introduced in 1955. It was not until 1965 that Gepts showed that major abnormalities occurred in beta cells in the islets of Langerhans in Type I diabetic patients. The availability of insulin radioimmunoassay (Yalow and Berson, 1960) and further research in the last two or three decades made it possible to differentiate Type I and Type II diabetes mellitus and to give the current definition of diabetes mellitus (for reviews, see Best, 1960 and Marble, 1971).

Diabetes mellitus is characterized by an absolute or relative deficiency of insulin and subsequent disorders in the metabolism of carbohydrate, fat and protein, as well as structural and functional abnormalities in a variety of tissues.

There are two common types of diabetes mellitus: Type I (insulin-dependent diabetes mellitus, IDDM), and type II (non-insulin-dependent diabetes mellitus, NIDDM). Three other types are also included in a new classification system adopted in 1979, which are: impaired glucose tolerance, gestational diabetes, and diabetes mellitus associated with other conditions or syndromes.

Table 1 summarizes two common types of diabetes mellitus, their etiology, epidemiology and clinical features. In type I, genetics, viruses and/or autoimmune factors have been suggested to be involved in the pathogenesis of the disease. Genetically, type I has been shown to be frequently associated with certain human leukocyte antigen (HLA) genes (Cudworth and Woodrow,
**TABLE I.**

**CHARACTERISTICS OF TYPE I AND TYPE II DIABETES MELLITUS**

<table>
<thead>
<tr>
<th></th>
<th>Type I (IDDM)</th>
<th>Type II (NIDDM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Etiology</strong></td>
<td>- genetic, viral or autoimmune abnormalities</td>
<td>- a decreased affinity of the receptors for insulin or a &quot;down regulation&quot; at insulin receptors, genetic or viral factors may be involved.</td>
</tr>
<tr>
<td><strong>Epidemiology</strong></td>
<td>- 5%-10% of all diabetics</td>
<td>- 90%-95% of all diabetics</td>
</tr>
<tr>
<td><strong>Clinical features</strong></td>
<td>- any age but usually &lt;30&lt;br&gt;- non-obese&lt;br&gt;- rapid onset&lt;br&gt;- ketosis common</td>
<td>- Any age but usually &gt;40&lt;br&gt;- 80% of type II obese&lt;br&gt;- slow onset&lt;br&gt;- ketosis rare except in stress or infection</td>
</tr>
<tr>
<td></td>
<td>- little or no endogenous insulin</td>
<td>- varying amounts of endogenous insulin, often higher than normal levels.</td>
</tr>
</tbody>
</table>
A diabetogenic variant of Coxsackie B4 virus has been identified to be able to damage the pancreatic beta cells (Yoon et al., 1979). Circulating islet cell antibodies capable of destroying beta cells in the pancreatic islets are found in type I diabetics (Lernmark et al., 1978). In type II, either a "post receptor defect", a decreased affinity of the receptors for insulin (Bar et al., 1978), or a "down regulation" of the insulin receptors as a consequence of increased levels of insulin (Kahn et al., 1977) are proposed to be possible factors in the pathogenesis of the disease.

Regardless of the etiology of diabetes mellitus, absolute or relative deficiency of insulin can result in abnormal metabolism of carbohydrate, protein and fat, leading to a series of clinical symptoms. Glucose uptake into muscle and adipose tissue, and glycogenesis in liver and muscle are reduced. At the same time, glycogenolysis and gluconeogenesis increase. All of these changes result in markedly elevated blood glucose levels and glucosuria occurs when the blood glucose levels exceed the renal threshold for glucose. Increased amounts of glucose in the urine causes osmotic diuresis giving rise to the symptom of polyuria, followed by polydipsia and polyphagia due to metabolic imbalance. Synthesis of fatty acids and triacylglycerol in liver and adipose tissue decreases and lipolysis in adipose tissue increases, resulting in hyperlipidemia. Insulin deficiency also reduces protein synthesis and weight loss follows (Ganong, 1985).

A large number of secondary complications are also associated with diabetes mellitus. Acute complications include ketoacidosis (McGarry and Foster, 1977) and non-ketotic diabetic coma (Arleff and Caroll, 1972). Much
more common now are chronic complications including microangiopathy which is probably responsible for diabetic retinopathy (Davis, 1974) and nephropathy (Balodimos, 1971); macroangiopathy which is perhaps related to atherosclerosis, coronary artery disease, myocardial infarction, hypertension and stroke (Renold et al., 1978); diabetic neuropathy (Sibley, 1982) and an increased incidence of urinary tract infections (Kass, 1956; Ooi et al., 1974). Diabetic cardiomyopathy is another complication whose importance has become recognized in recent years (Fein et al., 1980).

Most current evidence indicates that there is a common mechanism which induces these diverse complications of diabetes. It is suggested that insulin deficiency, hyperglycemia and other metabolic derangements play a central role in the pathogenesis of diabetic complications (Brownlee and Cerami, 1981; Gerich, 1986; Winegrad, 1987).

II. DIABETIC CARDIOMYOPATHY.

One of the major causes of morbidity and mortality in diabetics is cardiovascular disease, which accounts for about 80% of all diabetic deaths (Kannel, 1978). Besides coronary artery disease as a common complication, clinical (Ahmed et al., 1975; Regan et al., 1977), pathological (Hamby et al., 1974; Regan et al., 1977), epidemiological (Kannel et al., 1974), and experimental data (Regan et al., 1974; Fein et al., 1980) have strongly suggested that diabetes mellitus is associated with the development of a cardiomyopathy - diabetic cardiomyopathy. Diabetic cardiomyopathy is characterized as myocardial failure independent of atherosclerotic coronary
artery disease, valvular disease or hypertension.

Clinically, and pathologically, Hamby et al. (1974) reported evidence of myocardial dysfunction in the absence of large coronary disease in 16 diabetic patients and suggested that diabetic cardiomyopathy might be due to pathological changes in small coronary vessels. A shorter left ventricular ejection time, a longer pre-ejection period and a higher ratio of pre-ejection period/Left ventricular ejection time (PEP/LVET) were shown in diabetic subjects without myocardial ischemia or other cardiovascular disease (Ahmed et al., 1975). Post-mortem examination by Regan et al. (1977) showed nine out of eleven diabetics had no significant coronary disease, although six had bad heart failure at death. Shapiro et al. (1981) also observed an increased PEP/LVET ratio in diabetic patients. Furthermore, the Framingham study (Kannel et al., 1974) demonstrated that diabetic men had a two-fold increased risk and diabetic women a five-fold increased risk of congestive heart failure even without coronary or rheumatic heart disease present.

In experimental animal studies, a primary myocardial abnormality in diabetes was first shown in dog, a species known to have a low incidence of spontaneous arterial disease, by Regan and co-workers (1974). The end-diastolic volume and the stroke volume response were significantly less in eleven-month alloxan-diabetic dogs associated with a two-fold greater end-diastolic pressure increment, suggesting a decreased ventricular compliance in the absence of coronary artery disease. Similar abnormalities in ventricular function were reported in 18-month diabetic rhesus monkeys (Haider, et al., 1978). Studies on the effects of the diabetic state on myocardial mechanics (Fein et al., 1980) showed a slowing of relaxation as well as
a depression of shortening velocity in left ventricular papillary muscles from severely diabetic rats. Using the isolated working heart preparation, a decreased rate of contraction and relaxation at higher filling pressures (Penpargkul et al. 1980; Vadlamudi et al., 1982) and afterload (Ingebretson et al., 1980) have been reported. More recently, studies on papillary muscles from alloxan-diabetic rabbits demonstrated a markedly prolonged duration of isometric and isotonic contraction and relaxation (Fein et al. 1985).

The mechanisms involved in diabetic cardiomyopathy may include alterations in subcellular membranes and in a number of enzyme systems. These include depressed Ca$^{2+}$ stimulated ATPase activity in sarcoplasmic reticulum (SR) (Lopaschuk et al., 1983$^1$), lower oxidative phosphorylation capacity and Mg$^{2+}$-ATPase activity in mitochondria (Pierce and Dhalla, 1986), reduced Na$^+$,K$^+$-ATPase activities in sarcolemma (SL) (Ku and Sellers, 1982; Pierce and Dhalla, 1986). All of these changes result in a decreased capacity to transport Ca$^{2+}$ and subsequent depressed membrane function. An elevation in long chain acylcarnitine (LCAC) levels is correlated with the depression in Ca$^{2+}$-ATPase in SR (Adam et al., 1978; Lopaschuk et al., 1983$^1$) and Na$^+$, K$^+$-ATPase in SL (Wood et al., 1977). A depression in myocardial myosin and actomyosin ATPase has also been shown (Dillman, 1980; Malhotra et al., 1981), which may be due to changes in the myosin isozyme distribution (Dillman, 1980). In addition, myofibrillar basal (Mg$^{2+}$ dependent) and Ca$^{2+}$ stimulated ATPase activities in diabetic rat hearts were reported to be lower as compared to controls (Pierce and Dhalla, 1981). Other studies have shown a correlation between elevated levels of circulating and myocardial
fats and the development of diabetic cardiomyopathy (Rodrigues et al., 1985; Rodrigues et al., 1986; Heyliger et al., 1986).

III. DIABETES AND ALTERED LIPID METABOLISM IN THE HEART AND ADIPOSE TISSUE.

A number of experimental studies have implied a link between diabetes-induced metabolic changes and the development of cardiomyopathy (Regan et al., 1973; Shipp et al., 1973). One prominent metabolic change seen in diabetes is a decrease in glucose utilization by the heart due to lack of insulin. As a result, the heart derives the energy required for its mechanical activity almost entirely from lipid sources (Morgan et al., 1961; Randle et al., 1966).

Numerous alterations in the energy substrate metabolism pathways of diabetic myocardium have been documented. Glucose utilization is inhibited secondary to a decreased glucose transport. The increase in citrate levels due to increased fatty acid metabolism also decreases glycolysis by inhibiting phosphofructokinase (Randle et al., 1966). Increased fatty acid metabolism is associated with increased tissue levels of acyl CoA, acylcarnitine and triacylglycerols (fig. 1) (Denton and Randle, 1967; Feuvray et al., 1979).

Another biochemical abnormality in diabetes is a marked increase in adipose tissue lipolysis (McGarry and Foster, 1977). Triacylglycerol lipase is the rate-limiting enzyme in adipose tissue lipolysis. It is an insulin-inhibited, cyclic AMP-dependent enzyme; while catecholamines, glucagon and a number of other hormones can stimulate this enzyme resulting in hydrolysis of triacylglycerol accompanied by enhanced release of free
FIGURE 1

Possible metabolic changes in diabetes - arrow (↑,↓) indicates diabetes-induced changes.
fatty acids (FFA) (Steinberg, 1972; Fain, 1973). During insulin deficiency, triacylglycerol lipase is not inhibited and therefore increased FFA are produced. The fatty acids may diffuse out of the cell or they may be re-esterified into triacylglycerol within the adipocyte. The re-esterification process, however, requires alpha-glycerophosphate which is provided by insulin promoting the flux of glucose intracellularly. Thus, large quantities of FFA are released from the adipose tissue during insulin deficiency (Saudek and Eder, 1979). Diabetes is also associated with elevated plasma catecholamine levels (Paulson et al., 1980). Catecholamines bind to beta receptors of the adipocytes and increase cAMP which in turn leads to phosphorylation and activation of triacylglycerol lipase (William-Olsson et al., 1979). Hence, both lack of insulin and elevation of catecholamines contribute to the increased adipose tissue lipolysis in diabetes.

IV. TREATMENTS EMPLOYED TO AFFECT LIPID METABOLISM IN DIABETICS.

Since altered lipid metabolism in the diabetic myocardium may be involved in the diabetic cardiomyopathy, various attempts have been made to return lipid metabolism to normal in diabetic animals. Insulin treatment (9 U/kg/day) has been shown to normalize cardiac SR levels of long-chain acylcarnitines associated with a recovery of calcium transport activity. Heart function in diabetic rats treated with insulin was similar to control rat hearts (Lopaschuk et al., 1983). However, hypoglycemia remains a major clinical problem with insulin treatment. A more direct approach is carnitine treatment. Carnitine administration (3 g/kg/day) to diabetic rats effectively
prevented the accumulation of LCAC within the myocardium and the depression of calcium uptake in the SR. However, unlike insulin, carnitine was totally ineffective in improving cardiac function in diabetic rats (Lopaschuk et al., 1983). Another treatment that has been attempted in order to prevent lipid alterations is that of methyl palmoxirate. Methyl palmoxirate, a fatty acid analog, has been shown to inhibit fatty acid metabolism due to irreversible inhibition of carnitine acyl transferase, thus forcing carbohydrate utilization (Pearce et al., 1979). However, methyl palmoxirate treatment (25 mg/kg/day) was unable to control diabetes-induced changes in plasma glucose, insulin, lipids as well as functional myocardial depression; even though elevations of LCAC and depression of calcium uptake in SR of diabetic rat hearts could be prevented by the treatment (Tahiliani and McNeill, 1985). Hydralazine, a vasodilator used for the treatment of hypertension and congestive heart failure, has been shown to have the unusual effect of lowering blood lipids (Perry and Schroeder, 1955; Deming et al., 1958). Hydralazine treatment in diabetic animals also successfully prevented diabetes-induced hyperlipidemia. In addition, the functional depression seen in diabetic rat hearts was prevented by the treatment (Rodrigues et al., 1986). As myocardial lipid buildup has been suggested to be more closely related to the cardiac dysfunction associated with diabetes, further efforts have been made to control lipid metabolism in the diabetic myocardium. Choline and methionine, both of which are lipotropic agents (Young et al., 1956; Kuksis and Mookerjea, 1978), were reported to drastically reduce myocardial triacylglycerol and cholesterol levels in diabetic rats (Heyliger
et al., 1986). In addition, a significant improvement in heart function in treated diabetics was seen relative to the untreated ones.

V. MYO-INOSITOL (INOSITOL) AND DIABETES MELLITUS.

Myo-inositol is a dietary component as well as a precursor of cellular phosphatidylinositol. During the normal process of metabolism, myo-inositol is absorbed across the intestinal mucosa by a specific, Na⁺-dependent transport system (Caspary and Crane, 1970), circulated freely in plasma, filtered by the renal glomerulus and reabsorbed by a high-affinity Na⁺-dependent carrier-mediated transport system in the renal brush border membrane (Greene and Lattimer, 1986). Myo-inositol is also synthesized in many cells from glucose 6-phosphate by a specific cyclase and phosphatase (Burton and Wells, 1974; Chen and Eisenberg, 1975; Mauck et al., 1980). In the kidney, inositol is degraded to glucuronic acid by myo-inositol oxygenase (Howard and Anderson, 1967; Clements and Diethelm, 1979), while the major pathway for inositol metabolism in other tissues is reversible incorporation into the phosphoinositides, a specific class of membrane phospholipids, as shown in figure 2 (Berridge, 1985). Research suggests that the interaction of external agonists (e.g. alpha-1-adrenergic and muscarinic cholinergic agonists) with cell-surface receptors may stimulate phosphoinositide turnover in a variety of tissues and organ systems (Michell, 1975; Michell, 1979) including the heart (Quist, 1982; Brown et al., 1985). Two important second messengers are then released, inositol-1,4,5-trisphosphate (IP₃) which has been implicated in the release
FIGURE 2.
MYO-INOSITOL METABOLISM CYCLE

EXTERNAL AGONISTS
e.g. \( \text{\textalpha_1-ADRENERGIC OR MUSCARINIC CHOLINERGIC AGONISTS} \)

\[ \text{PHOSPHOLIPASE C} \]

\[ \text{INOSITOL TRISPHOSPHATE (IP}_3^\text{)} \]
\[ \text{INOSITOL BISPHOSPHATE (IP}_2^\text{)} \]
\[ \text{INOSITOL PHOSPHATE (IP}_1^\text{)} \]

\[ \text{PHOSPHATIDYLINOSITOL 4,5 BISPHOSPHATE (PIP}_2^\text{)} \]
\[ \text{PHOSPHATIDYLINOSITOL 4-PHOSPHATE (PIP)} \]

\[ \text{DIACYLGLYCEROL (DG)} \]
\[ \text{PHOSPHATIDIC ACID (PA)} \]

\[ \text{PHOSPHATIDYLINOSITOL (PI)} \]

\[ \text{MYO-INOSITOL} \]
of calcium from intracellular storage sites in many tissues (Streb et al., 1983; Berridge, 1984; Berridge and Irvine, 1984) and diacylglycerol (DG) which can activate protein kinase C and result in phosphorylating specific membrane proteins (Nishizuka, 1983; Nishizuka, 1984; Haslam and Davidson, 1984). There is some evidence that IP$_3$ can mobilize calcium in the heart (Hirata et al., 1984; Fabiato, 1986; Nosek et al., 1986), and it is proposed that IP$_3$ may be involved in excitation-contraction coupling in cardiac muscle (Poggioli et al., 1986).

Myo-inositol was first shown to be a lipotropic agent in various animal species in 1941 when Gavin and McHenry reported that inositol could prevent the development of the fatty liver and the accumulation of cholesterol in liver. Subsequently Dotti et al. (1951) reported that 0.5 g of inositol daily added to the high cholesterol diet eliminated the expected rise in blood cholesterol and phospholipid in rabbits. Liver triacylglycerols were shown to accumulate in experimental animals under conditions of inositol deficiency (Hayashi et al., 1974).

Research on the relationship between diabetes mellitus and myo-inositol metabolism goes back to 1858 when Vohl first discovered that human diabetics excreted large amounts of myo-inositol in their urine. This was confirmed by Freinkel et al. (1960) and Pitkanen (1972). The inosituria in diabetics may result from the competitively inhibitory effect of hyperglycemia on renal tubular reabsorption (Clements and Diethelm, 1979; Hammerman et al., 1980) or the inhibitory effect of diabetes on the activity of inositol oxygenase, the enzyme responsible for metabolizing inositol in
the kidney (Whiting et al., 1979). Furthermore, Clements and Reynertson (1977) showed that a widespread intracellular deficiency of myo-inositol was present in untreated diabetics. The deficiency was shown to selectively exist in some tissues in which glucose transport is not rate-limiting for its metabolism, such as peripheral nerve (Greene et al., 1975), retina (MacGregor et al., 1984), arterial wall (Morrison, 1984) and renal glomerulus (Beger-Mears et al., 1984). However, plasma inositol was significantly higher in uncontrolled diabetics which may be due to enhanced gastrointestinal absorption and impaired intracellular transport, both of which may result from hyperglycemia as insulin treatment could correct these abnormalities (Clements and Reynertson, 1977).

Myo-inositol has been used in the treatment of diabetics. Clements and Reynertson (1977) showed that a 3-g oral load of inositol could significantly elevate plasma inositol concentrations in human subjects with diabetics showing a greater response. The administration of inositol, 0.5 g twice daily for two weeks, has been reported to be beneficial in the treatment of diabetic neuropathy (Greene et al., 1975; Salway et al., 1978) through increased Na\(^+\)-K\(^+\)-ATPase activity with resulting improvement of nerve conduction velocity (Green and Lattimer, 1983).

The above observations suggest that myo-inositol may play a role in regulating lipid metabolism and may be beneficial in the prevention and treatment of cardiomyopathy associated with diabetes mellitus. We therefore investigated the effect of myo-inositol on elevated plasma and myocardial lipids and depressed cardiac performance in diabetic rats.
VI. THYROID HORMONES.

Thyroid hormones, including thyroxine (T\textsubscript{4}) and 3,5,3'-triiodothyronine (T\textsubscript{3}), are produced and secreted under stimulation of thyroid-stimulating hormone (TSH) from the anterior pituitary, which, in turn, is stimulated by thyrotropin-releasing hormone (TRH) from the hypothalamus. A negative feedback mechanism operates to decrease the release of TSH in the presence of high levels of T\textsubscript{3} or T\textsubscript{4} (Spaulding and Utiger, 1981). Thyroxine is degraded by monodeiodination to form either T\textsubscript{3} (Pitt-Rivers, 1955; Braverman, 1970) or 3,3',5'-triiodothyronine (reverse T\textsubscript{3}, rT\textsubscript{3}) (Flock, 1961; Gavin, 1977). T\textsubscript{3} is biologically more potent than T\textsubscript{4}, while rT\textsubscript{3} has insignificant biological activity (Spaulding and Utiger, 1981).

Diabetes mellitus is a metabolic disorder which is related to the alterations not only in insulin levels but also in levels of several other hormones (Johansen and Hansen, 1969; Lefebvre and Luyckx, 1979; Tzagournis, 1982) including thyroid hormones (Cooppan, 1982). Plasma thyroid hormones have been reported to be normal (Pittman et al., 1979\textsuperscript{2}), slightly reduced (Naeije et al., 1978) or substantially reduced in uncontrolled diabetic patients (Saunders et al., 1978). Studies with animal models of chemically-induced diabetes have shown decreased plasma T\textsubscript{4} and T\textsubscript{3} levels in diabetic rats (Zaninovich et al., 1977; Boado et al., 1978). Extrathyroidal conversion of T\textsubscript{4} to T\textsubscript{3} in diabetic rats was reduced (Balsam et al., 1978; Pittman et al., 1979\textsuperscript{2}), and was correlated with reduced activity of T\textsubscript{4}-5'-deiodinase in the liver of diabetic rats (Gavin et al., 1981). In addition,
deficient TSH secretion has been demonstrated in diabetic animals during a low iodine diet regime (Periscas and Jolin, 1977). Furthermore, Wilber et al. (1981) found that diabetes mellitus is also associated with a reduction in circulating TRH.

Various suggestions regarding the mechanisms of these reductions have been proposed. Reduced production of $T_3$ from $T_4$ may be related to decreased glucose uptake and utilization (Saunders et al., 1978), as insulin therapy improved the conversion from $T_4$ to $T_3$ which correlated well with the normal level of blood glucose (Pittman et al., 1979). Changes in TSH secretion could result from the interference with the normal negative feedback mechanism, either by altering the relationship of free and protein bound circulating thyroid hormone due to the increased FFA displacing thyroid hormone from their protein binding (Liewendahl and Helenius, 1976), or by changing the utilization and disposal of the thyroid hormones by peripheral tissues. Moreover, diabetes may directly influence pituitary TSH secretion by some localized metabolic effect on thyrotroph cells. This is supported by the histological findings of Desclaux et al. (1948), who observed degranulation of the acidophil cells and a decreased number of basophil cells in the pituitary of alloxan-diabetic rats. Furthermore, the increase in the pituitary-adrenal axial activity in diabetic rats and mice (L'Age et al., 1940; Coleman & Burkart, 1977) may result in a decreased TSH secretion (Sakiz and Guillemin, 1965). Finally, the reduced TSH secretion may be secondary to lowered hypothalamic secretion of TRH. As discussed above, a significant decrease of circulating TRH was observed in diabetes (Wilber
et al., 1981). The mechanism(s) by which the diabetic state affects TRH secretion remains to be investigated.

The amount of circulating thyroid hormones has profound effects on the responsiveness of the cardiovascular system. There is evidence that thyroid hormones have a direct positive inotropic effect on the heart (Markowitz and Yater, 1932), which is exerted by binding with myocardial nuclear receptors and subsequent promotion of the synthesis of RNA and protein (Tata and Windell, 1966; Surks et al., 1973; Tsai and Samuels, 1974; Carter et al., 1985). Philipson and Edelman (1977) have documented that the activity of Na\(^+\)-K\(^+\)-ATPase in the heart is increased when T\(_3\) is administered to hypothyroid rats. The activity of the SR Ca\(^{2+}\)-stimulated ATPase has also been shown to be increased (Suko, 1973; Limas, 1978). Another important enzyme, whose activity is controlled by the level of thyroid hormone and is related to the positive inotropic effect of thyroid hormone, is ventricular myosin ATPase. The myosin ATPase has been shown to exist in three isozymic forms (V\(_1\), V\(_2\), V\(_3\)) in order of decreasing electrophoretic mobility and ATPase activity in rats, rabbits and a number of other species (Hoh et al., 1977; Morkin et al., 1983). In the adult euthyroid state, V\(_1\) strongly predominates in rats (85%) while in rabbits V\(_3\) represents 85% of the total enzyme (Lompre, et al., 1981). The relative proportions of these isozymic forms in rats and rabbits have been shown to depend upon the function of the thyroid. In general, thyroid hormone enhances the synthesis of the V\(_1\) form which results in increased myosin ATPase activity (Hoh et al., 1977; Martin, et al., 1982). In man the myosin
Isozyme distribution patterns were suggested to be similar to rabbits but the effects of thyroid hormone on the patterns were relatively less (Morkin et al., 1983).

Thyroid hormone exerts its effect on the heart not only directly but also indirectly through adrenergic stimulation (Graves, 1835; Levey, 1971). An increased number of beta-adrenergic binding sites have been shown in membranes from hyperthyroid hearts while the affinity of the receptor for catecholamines remained unchanged (Williams et al., 1977; Banerjee and Kung, 1977; Ciaraldi and Marinetti, 1977). However, the number of alpha-adrenergic receptors was decreased (Sharma and Banerjee, 1978; Williams and Lefkowitz, 1979).

Diabetes-induced hypothyroidism has been shown to result in altered myosin isoenzyme distribution (from V₁ to V₃) (Dillman, 1980). The V₃ form is the slowest to hydrolyze ATP and to form cross bridges. The predominance of the V₃ form (about 68% of the total in diabetic rats) would therefore result in a reduced myosin ATPase activity.

Thus, it appears that thyroid hormones play some role in the regulation of myocardial contractility. In addition, diabetes is associated with a hypothyroid state. It was therefore of interest for us to investigate the effect of both T₃ and myo-inositol on the cardiac dysfunction of diabetic rats.

**Specific Goals of the Present Investigation.**

1. To determine if plasma and myocardial triacylglycerols, cholesterol and
phospholipid levels are elevated in streptozotocin (STZ)-diabetic rats.

2. To study the role of elevated myocardial lipid levels in the development of diabetic cardiomyopathy.

3. To investigate the effect of myo-inositol on cardiac dysfunction and elevated myocardial lipid levels in diabetic rats.

4. To determine if myo-inositol plus T₃ treatment could further improve heart function and myocardial lipid levels in STZ diabetic rats.
MATERIALS AND METHODS

1. MATERIALS

1. ANIMALS.

Female Wistar rats weighing between 190-215 g were used throughout the study. The rats were obtained from U.B.C. Animal Care facilities.

2. CHEMICALS

The following chemicals were purchased from Sigma Chemical Co.: Bovine Serum Albumin, citric acid, copper sulfate, ether, Folin-Ciocalteu's phenol reagent, myo-inositol, sodium bicarbonate, sodium potassium tartrate, streptozotocin (STZ), sucrose, L-3,5,3'-triiodothyronine, Tris-base.

Calcium chloride, chloroform, D-glucose, hydrochloric acid, magnesium chloride, methanol, potassium chloride and sodium chloride were purchased from BDH.

3. ASSAY KITS.

Insulin radioimmunoassay (RIA) kits, Amerlex T₃ RIA kits and Amerlex T₄ RIA kits were purchased from Amersham.

Glucose assay kits, cholesterol assay kits, phospholipid assay kits and triacylglycerol assay kits were purchased from Boehringer-Mannheim.
II. METHODS

(1) INDUCTION OF EXPERIMENTAL DIABETES.

Female Wistar rats between 190-215 g were used. All animals were anesthetized with ether prior to injection of either STZ or its vehicle into the tail vein. Diabetes was induced by a single intravenous injection of STZ (55 mg/kg) dissolved in 0.1 M citrate buffer (pH 4.5). Control rats were injected with citrate buffer alone. All rats injected with STZ survived and were housed three to four per cage. Food and water were provided ad libitum throughout the study period. Diabetes was detected three days later by estimating the extent of glucosuria with the aid of Lilly Tes-tape.

2. TREATMENT PROTOCOLS.

1. MYO-INOSITOL PRELIMINARY STUDY.

Three days after STZ injection, the diabetic rats were randomly divided into six groups: untreated diabetic, myo-inositol-treated diabetic (with myo-inositol concentrations of 2, 3, 4, 6 or 8 mg/ml respectively). Myo-inositol was added to the drinking water of the treated animals. Treatment was carried out for 8 weeks and at the end of this period the animals were sacrificed. Blood samples were collected in heparinized tubes, centrifuged at 1300 g for 20 min to separate cells from plasma. The plasma obtained was stored at -20°C until assayed. Plasma was analyzed
for glucose, cholesterol and triacylglycerols. Hearts were immediately frozen with a Wollenberger clamp previously cooled in liquid nitrogen and stored at -25°C for the determination of myocardial cholesterol and triacylglycerols.

(2) MYO-INOSITOL TREATMENT STUDY.

Control and diabetic rats were subdivided into two groups, each at random, 3 days after diabetes induction. Each group drank one of the following fluids: 1) control group: water; 2) control with myo-inositol-treatment group: water containing myo-inositol, 3) diabetic group: water; 4) diabetic with myo-inositol treatment group: water containing myo-inositol. For diabetic rats, the concentration of myo-inositol given was 3.5 mg/ml, which was determined from the preliminary study as the most effective concentration to treat diabetics. Controls received concentrations three times higher because their fluid intake was approximately three times lower than that of diabetics. The concentration of myo-inositol in water was adjusted to give the same dose of myo-inositol (2.5 g/kg/day) to controls and diabetics. The intake of agents for each group (mg/kg \(^{-1}\)/day \(^{-1}\)) was calculated with the following formula:

\[
\text{Intake (mg/kg}^{-1}\text{/day}^{-1}) = \frac{\text{concentrations of agent (mg/ml)}}{\text{body weight (g)}} \times 1000
\]

All animals were sacrificed after eight weeks of treatment. Hearts were excised quickly and perfused in the working heart mode as described below and then frozen with a Wollenberger clamp previously cooled in liquid nitrogen and stored at -25°C for the determination of myocardial lipids.
In the myocardial lipid study, triacylglycerols, cholesterol and phospholipid were measured. Blood was collected in heparinized tubes, centrifuged at 1300 g for 20 min. The plasma obtained was stored at -20°C until assayed for glucose, insulin, T₃, T₄, triacylglycerols, cholesterol and phospholipids.

(3) MYO-INOSITOL AND TRIIODOTHYRONINE (T₃) TREATMENT STUDY.

Control and diabetic rats were randomly divided into eight groups: controls; myo-inositol-treated controls; T₃-treated controls; myo-inositol plus T₃-treated controls: diabetic; myo-inositol-treated diabetic; T₃-treated diabetic; and myo-inositol plus T₃-treated diabetic. Myo-inositol at a dose of 2.5 g/kg/day was administered in the drinking water to myo-inositol-treated and myo-inositol plus T₃-treated control or diabetic rats. The intake of agents for each group (mg/kg·day⁻¹) was calculated with the same formula as shown in the "myo-inositol treatment study" part. This myo-inositol dose was chosen as our myo-inositol treatment study had shown that it could decrease myocardial triacylglycerol, cholesterol and phospholipid levels and partially improve cardiac function in diabetic rats. T₃ at a dose of 30 μg/kg·day⁻¹ was injected subcutaneously to T₃-treated and myo-inositol plus T₃-treated control or diabetic rats. This dose was selected as previous studies have shown that it can effectively prevent the depression of myosin ATPase (Dillmann, 1982). Rats were treated for eight weeks and then decapitated. Blood was collected in heparinized tubes, then centrifuged at 1300 x g for 20 min. The plasma obtained was stored at -20°C for the later determination of glucose, insulin, T₃, T₄, triacylglycerols, cholesterol and phospholipid levels. Hearts were perfused in the
working heart mode and then stored at -24°C for myocardial lipid measurement.

3. ISOLATED WORKING HEART PERFUSION

Cardiac performance was assessed using a modification of Neely's isolated working heart preparation, as described by Vadlamudi et al. (1982). The perfusion fluid used was Chenoweth-Koelle (CK) buffer of the following composition (millimolar concentrations of solutes): NaCl, 120; KCl, 5.6; CaCl$_2$, 2.18; MgCl$_2$, 2.1; NaHCO$_3$, 19; glucose 10. The CK buffer was oxygenated continuously with a 95% O$_2$ and 5% CO$_2$ mixture. The pH of the buffer was maintained at 7.4, and temperature kept constant at 37°C±1°C. Rats were sacrificed by decapitation with a guillotine. Hearts were quickly excised from the rats and placed in warm aerated CK buffer where extraneous tissue was dissected free. The aortic stump was located and tied to a 15-gauge stainless steel aortic perfusion cannula. Perfusion was initiated in the retrograde manner through the aorta at 45 cm H$_2$O (30 mm Hg) aortic filling pressure. The left atrium was then attached through the pulmonary vein to a 16-gauge stainless steel cannula which was connected to atrial filling reservoirs. Cardiac work was started by switching the perfusion system from the retrograde mode to the working heart mode. In the working heart mode, the perfusate entered the left ventricle through the left atrium and was pumped out into the aortic stump. The aortic outflow was connected to a compliance chamber containing 2-3 ml of air. Hearts were subjected to an afterload of 75 cm H$_2$O. LVDP was measured
by means of a Statham P23AA transducer (Statham-Gould Instruments) attached to a 20-gauge needle through a 3 cm piece of polyethylene (PE-90) tubing. The needle was inserted through the apex of the heart into the left ventricle. Intraaortic pressure was measured by means of another Statham P23AA transducer attached to a sidearm of the aortic outflow system. LVDP, intraaortic pressure and the first derivative of LVDP (positive and negative dP/dt) were recorded on a Grass Model 79D polygraph. The hearts were stimulated by means of 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 300 beats/min. Data were collected and analyzed with a microcomputer according to the method of Harris et al. (1983). The pressure transducer signal from the polygraph was sampled at 667 Hz over 1.5 seconds at each function point. This resulted in data being collected for six complete cardiac pulses and three of these were analyzed by use of curve-fitting techniques to determine pulse height, area, start and finish. Values from these three pulses were averaged to produce data values at each function point.

The hearts were equilibrated at 15 cm H\textsubscript{2}O atrial filling pressure for about 10 min before function curves were performed. Studies were performed by estimating the left ventricular function against varying left atrial filling pressures (through varying the height of the left atrial filling reservoir) from 7.5 cm H\textsubscript{2}O to 20.0 cm H\textsubscript{2}O in 2.5 cm steps. The filling pressure was first reduced stepwise from 15.0 to 7.5 cm H\textsubscript{2}O, then increased stepwise to 20.0 cm H\textsubscript{2}O and finally decreased stepwise to 15.0 cm H\textsubscript{2}O.
At each point, pressure development was allowed to stabilize before it was recorded. In general, stable pressure development was achieved within 2 min after the left atrial filling pressure was changed. A complete function curve took about 20-30 min and the total time of perfusion was approximately 40 min.

4. MEASUREMENT OF PLASMA TRIACYLGlycerols, CHOLESTEROL AND PHOSPHOLIPID LEVELS

Plasma triacylglycerols, cholesterol and phospholipids were determined using the respective Boehringer-Mannheim diagnostic reagent kits, all of which rely on enzymatic reactions followed by colorimetric determinations.

5. DETERMINATION OF MYOCARDIAL TRIACYLGlycerols, CHOLESTEROL AND PHOSPHOLIPID LEVELS.

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 homogenizer (3x20 s; setting 5). An aliquot (1.0 ml) of this homogenate was used for lipid analysis. Lipids were extracted from heart homogenates with a 2:1 chloroform-methanol solution for 3 hours. After aspirating the methanol and removing the denatured protein that precipitated at the chloroform-methanol interface, a 400 ul aliquot of the remaining chloroform in which the lipids were dissolved was evaporated in a stream of nitrogen. The residue remaining was then assayed for cholesterol, phospholipids and triacylglycerols using
the respective kits from Boehringer Mannheim Canada Inc.

6. PROTEIN ASSAY

The protein in the homogenate was quantified using the standard Lowry (1951) protein assay. Bovine serum albumin was used as a standard. A 25 ul aliquot of the ventricular tissue homogenate was added to 0.975 ml of distilled water. To this was added 5.0 ml of copper reagent (1 ml 2% Na$_2$CO$_3$ in 0.1 N NaOH. 0.01 ml 2% Na$^+$.K$^+$ tartrate and 0.01 ml 1% CuSO$_4$ per ml of reagent), the tubes were vortexed and incubated at room temperature for 10 minutes. This was followed by the addition of 0.5 ml of a 50/50 Folin-Ciocalteu's phenol reagent/water mixture. Thirty min after adding copper reagent, the absorbance was read at 623 nm.

7. BLOOD ANALYSIS

(1) Plasma glucose was determined by the glucose oxidase method using a Boehringer-Mannheim glucose kit.

(2) Plasma immunoreactive insulin was assayed by the radioimmunoassay method of Herbert et al. (1965) using the Amersham insulin radioimmunoassay (RIA) kit. The insulin assay reagents consisted of human insulin standards, [$^{125}$I] porcine insulin and an insulin antibody raised against porcine insulin, for which human insulin exhibits 100% and rat insulin 90% cross reactivity. Human insulin standards were used since the error made in the estimation of rat insulin levels was minimal due to the very high cross reactivity
with rat insulin (about 90%).

(3) Plasma triiodothyronine (T₃) and thyroxine (T₄) levels were measured using the Amerlex T-3 and T-4 radioimmunoassay (RIA) kits of Amersham.

8. STATISTICAL ANALYSIS

The data are expressed as mean±standard error of the mean (S.E.M.). Statistical significance was determined by a two way analysis of variance followed by a Newman-Keul's test. The level of statistical significance was set at a probability of less than 0.05 (p<0.05).
RESULTS

A. PRELIMINARY STUDY

In the preliminary study, the most effective concentration of MI in decreasing lipid levels in the diabetic rats was determined. Diabetes was induced by injection with STZ (55 mg/kg, i.v.). Three days after diabetes induction, myo-inositol was administered at different concentrations (2, 3, 4, 6, 8 mg/ml) in the drinking water and treatment continued for 8 weeks. Results showed that plasma glucose, plasma and myocardial triacylglycerol levels tended to decrease in diabetics treated with myo-inositol at concentrations of 3 and 4 mg/ml (table 2). There was no significant difference in plasma and myocardial cholesterol among all six groups.

B. MYO-INOSITOL TREATMENT STUDY

1. EFFECT OF STZ-INDUCED DIABETES ON CARDIAC FUNCTION, PLASMA AND MYOCARDIAL LIPID LEVELS IN FEMALE WISTAR RATS

In all of our studies, diabetes was induced by a single i.v. injection of 55 mg/kg STZ. The general features of STZ-diabetic rats were quite characteristic of the disease and are summarized in table 3. Body weight gain during the study period was significantly less in the diabetic groups than in the control rats so that the diabetic rats had significantly lower body weights upon sacrifice. Plasma glucose levels measured at the time of sacrifice were significantly higher in the STZ-injected rats accompanied by a marked depression in plasma insulin levels. An elevated urine glucose
(>2%) was observed in diabetic rats throughout the study period. In addition, during the study period the diabetic rats exhibited polyuria, polydipsia (table 4) and polyphagia. When thyroid status of experimental rats were measured, it was observed that plasma T3 and T4 levels were significantly lower in the untreated diabetic rats compared to controls (table 5).

Plasma lipid profiles in the experimental rats are shown in figures 3-5. Plasma triacylglycerols (figure 3), cholesterol (figure 4) and phospholipid (figure 5) were markedly elevated in the untreated diabetic group relative to controls.

Myocardial triacylglycerols (figure 6), cholesterol (figure 7) and phospholipid (figure 8) were elevated in untreated diabetic rats relative to controls.

When the hearts were isolated and perfused in the working heart mode, it was observed that cardiac performance assessed by measuring LVDP, rate of pressure development (+dP/dt) and rate of pressure decline (-dP/dt) was significantly depressed in 8-week diabetic rats at all filling pressures (figures 9-11) compared to controls.

2. EFFECT OF MYO-INOSITOL TREATMENT ON CARDIAC DYSFUNCTION AND ELEVATED PLASMA AND MYOCARDIAL LIPID LEVELS IN STZ-DIABETIC RATS.

Myo-inositol treatment in control and diabetic rats was initiated three days after injecting them with buffer or STZ (55 mg/kg i.v.) and was continued for 8 weeks. There was no significant difference between body weight gain, fluid intake, plasma glucose, plasma insulin, plasma T3 or T4 of untreated and myo-inositol treated controls (table 3-5). Both untreated
and treated diabetic rats gained less weight than the controls. In addition, both untreated and treated diabetic rats had significantly higher fluid intake, markedly elevated blood glucose levels and severe hypoinsulinemia (table 3 and 4). Therefore it seemed that myo-inositol treatment did not alter the diabetic status of rats. T₃ and T₄ levels of the diabetic rats were significantly lower than controls, and myo-inositol treatment did not affect T₃ and T₄ levels in diabetic rats (table 5).

Plasma triacylglycerols (figure 3), cholesterol (figure 4) and phospholipid (figure 5) were markedly elevated in the untreated diabetic group. In contrast, myo-inositol treatment of the diabetic rats restored plasma triacylglycerols, cholesterol and phospholipids back to normal, while the treated controls did not exhibit lowered plasma triacylglycerols, cholesterol and phospholipid levels compared to untreated controls.

Triacylglycerols, cholesterol and phospholipid were increased in the myocardium of untreated diabetics (figure 6-8). Supplementation with myo-inositol also affected the diabetic myocardial lipid levels. The triacylglycerols content of the treated STZ animals was significantly lower than that of the untreated diabetics and was even lower than untreated controls (figure 6). Myocardial triacylglycerols of treated controls were also reduced relative to the untreated controls. Myocardial cholesterol was also reduced in treated diabetics relative to their untreated counterparts (figure 7). Myocardial cholesterol was similar in both untreated and treated controls. Myocardial phospholipid of treated diabetics was significantly lower than that of untreated diabetics, but was still higher than both
controls (figure 7). Hence it seems that myo-inositol controlled the elevated myocardial triacylglycerols, cholesterol and phospholipid of the diabetic rats. Myocardial triacylglycerols of the myo-inositol-treated controls also appeared to be affected.

Figures 9-11 show the effect of myo-inositol treatment on diabetes-induced depression of LVDP, +dP/dt and -dP/dt. Hearts from untreated diabetic rats showed lower LVDP, +dP/dt and -dP/dt compared to both untreated and treated controls at all filling pressures examined (from 7.5 cm H$_2$O to 20.5 cm H$_2$O). There was no significant difference in LVDP, +dP/dt and -dP/dt between untreated and treated controls. Treatment of the diabetic rats with myo-inositol did not appear to have any significant effect on LVDP at 7.5 cm H$_2$O of filling pressure (figure 9). At filling pressure more than 10 cm H$_2$O, the pressure developed in myo-inositol treated diabetics was significantly higher than untreated diabetics but was still significantly lower than both controls. Similar results were also seen for the rate of pressure development (+dP/dt) (figure 10). However, in the case of treated diabetics, the +dP/dt at 7.5 cm H$_2$O of filling pressure was partially improved and +dP/dt at 17.5 cm H$_2$O of filling pressure was not different from untreated diabetics. For the rate of pressure decline (-dP/dt) (figure 11), myo-inositol treatment of the diabetic rats restored the depressed -dP/dt back to normal at filling pressures of 10 cm H$_2$O and 12.5 cm H$_2$O, partially reversed this depressed parameter at filling pressures of 7.5 cm H$_2$O and 15 cm H$_2$O, and had no effect on -dP/dt at filling pressures greater than 17.5 cm H$_2$O. These results
confirm the observation that untreated diabetic rats are characterized by a depression of cardiac function (Penpargkul et al., 1980; Vadlamudi et al., 1982). Myo-inositol treatment partially improved the cardiac function of STZ-diabetic rats.

C. MYO-INOSITOL AND/OR T₃ TREATMENT STUDY.

1. EFFECT OF STZ-INDUCED DIABETES ON CARDIAC FUNCTION, PLASMA AND MYOCARDIAL LIPID LEVELS IN FEMALE WISTAR RATS.

The general features of STZ (55 mg/kg, i.v.)-diabetic rats are summarized in table 6. The diabetic rats exhibited reduced body weights compared to all controls. Plasma glucose levels were markedly elevated in STZ-injected rats accompanied by a depression in plasma insulin levels. An elevated urine glucose (>2%) was observed in diabetic rats throughout the study period. In addition, the diabetic rats exhibited polyuria, polydipsia (table 7) and polyphagia during the 8 week study period. When thyroid status was studied, untreated diabetics exhibited lower plasma T₃ and T₄ levels compared to controls (table 8).

Figures 12-14 show the plasma lipid profiles in the experimental rats. Plasma triacylglycerols (figure 12), cholesterol (figure 13) and phospholipids (figure 14) were significantly higher in the untreated diabetic rats compared to all controls.

Myocardial triacylglycerols (figure 15), cholesterol (figure 16) and phospholipids (figure 17) were also elevated in untreated diabetic rats relative
Hearts obtained from rats diabetic for eight weeks exhibited depressed cardiac function. The LVDP, +dP/dt and -dP/dt were all significantly depressed in the diabetic hearts at all filling pressures studied (figure 18-20).

2. EFFECT OF MYO-INOSITOL AND/OR T₃ TREATMENT ON CARDIAC DYSFUNCTION AND ELEVATED PLASMA AND MYOCARDIAL LIPID LEVELS IN STZ-DIABETIC RATS.

Myo-inositol (2.5 g/kg/day) and/or T₃ (30 ug/kg/day) treatment in control and diabetic rats were initiated three days after injecting rats with buffer or STZ (55 mg/kg, i.v.) and was continued for 8 weeks. There was no significant difference in body weight gain, fluid intake, plasma glucose, plasma insulin, and plasma T₃ among untreated control, myo-inositol-treated control, T₃-treated control and myo-inositol plus T₃-treated control groups (tables 6-8). Untreated diabetic rats gained weight significantly less than all controls while myo-inositol and/or T₃ treatment had no effect on the body weight gain in diabetic rats (table 6). Fluid intake was markedly elevated in the untreated diabetic rats. Myo-inositol treated diabetic rats had lower fluid intake than untreated diabetics, but still higher than controls (table 7). T₃-treated and myo-inositol plus T₃-treated diabetic animals had even higher fluid intake than untreated diabetics. Untreated diabetic rats exhibited severe hyperglycemia and hypoinsulinemia. Myo-inositol and/or T₃ treatment did not affect plasma glucose and plasma insulin levels in diabetic rats (table 6). It thus seemed that the diabetic status of rats was
not altered by either myo-inositol or \( T_3 \) treatment, or both. Plasma \( T_3 \) levels of the diabetic rats were significantly lower compared to all controls. \( T_3 \) alone or myo-inositol plus \( T_3 \)-treatment reversed \( T_3 \) levels back to normal in diabetic rats, whereas myo-inositol treated diabetic rats still had lower \( T_3 \) values relative to controls. While diabetic rats had lower \( T_4 \) values than controls, all animals treated with \( T_3 \) (controls and diabetics) had significantly lower values as compared with the respective untreated controls or diabetics.

Plasma triacylglycerols, cholesterol and phospholipids were markedly elevated in the untreated diabetics compared to all controls (figures 12-14). Myo-inositol treatment alone restored the plasma triacylglycerols, cholesterol and phospholipids of diabetic rats back to normal. \( T_3 \) treatment alone and myo-inositol plus \( T_3 \) treatment could not lower plasma triacylglycerols and cholesterol levels of diabetic rats. Plasma phospholipids of diabetic rats were not affected by myo-inositol plus \( T_3 \) treatment and was further increased with \( T_3 \) treatment alone. Plasma triacylglycerols, cholesterol and phospholipids of control rats were not affected by either myo-inositol or \( T_3 \) treatment or both. Hence it seems that myo-inositol treatment alone controlled elevated plasma lipids of the diabetic rats having no effect in the controls. \( T_3 \) treatment or the combination treatment did not affect or further increased the plasma lipids of the diabetic rats.

Myocardial triacylglycerols, cholesterol and phospholipids of untreated diabetic rats were markedly elevated compared to all control rats (figures 15-17). Myocardial triacylglycerols, cholesterol and phospholipid contents
were similar in controls and diabetics treated with myo-inositol alone. Myocardial triacylglycerols and phospholipid concentrations of diabetic rats were not affected by T₃ treatment alone, while myocardial cholesterol was further increased with T₃ treatment alone relative to untreated diabetics. Myocardial triacylglycerols of diabetic rats were lowered by myo-inositol plus T₃ treatment, but still higher than controls. Myocardial cholesterol and phospholipids were further increased with myo-inositol plus T₃ treatment compared to untreated diabetics. Myocardial lipids of control rats appeared not to be affected by myo-inositol and/or T₃ treatment. This study shows that myo-inositol treatment alone controlled elevated myocardial lipids of STZ diabetic rats. T₃ treatment or myo-inositol plus T₃ treatment did not affect or further increased myocardial lipids of diabetic rats. Neither myo-inositol or T₃ or both had any effect in the controls.

Figures 18-20 show the effect of myo-inositol and/or T₃ treatment on diabetes-induced depression of LVDP, +dP/dt and -dP/dt. Hearts from untreated diabetic rats showed lower LVDP, +dP/dt and -dP/dt compared to untreated controls at all filling pressures. Myo-inositol treatment alone in the diabetic rats restored the LVDP back to normal only at 7.5 cm H₂O of filling pressure (figure 18). At filling pressures greater than 10 cm H₂O, the pressure developed in myo-inositol treated diabetics was significantly higher than untreated diabetics but was still significantly lower than untreated and myo-inositol treated controls. LVDP was significantly higher in the myo-inositol treated controls relative to their untreated counterparts at filling pressures greater than 12.5 cm H₂O. In the T₃ treated
controls or diabetics no significant elevation or depression of LVDP was seen as compared to untreated controls or diabetics, respectively. Myo-inositol plus T₃ treatment of the diabetic rats restored LVDP to normal at 7.5 cm H₂O of filling pressure, and partially improved LVDP at filling pressures greater than 10.0 cm H₂O. However, at filling pressures greater than 12.5 cm H₂O, myo-inositol plus T₃ treated diabetic rats had lower LVDP values as compared to myo-inositol treated diabetic rats. LVDP in hearts from myo-inositol plus T₃ treated controls did not differ significantly from untreated controls. For the rate of pressure development (+dP/dt) (figure 19), there was no significant difference among untreated, myo-inositol treated, T₃-treated and myo-inositol plus T₃-treated controls. Myo-inositol pretreatment of the diabetic rats partially reversed the depression in +dP/dt at all filling pressures. T₃ treatment did not appear to have any significant effect on +dP/dt in diabetic rats. Myo-inositol plus T₃ treatment of the diabetic rats partially improved +dP/dt at all filling pressures. However, at filling pressures greater than 17.5 cm H₂O, myo-inositol plus T₃ treated diabetic rats had lower +dP/dt values relative to myo-inositol treated diabetic rats. Similar results were also seen for the rate of pressure decline (-dP/dt) (figure 19). However, in this case, myo-inositol treated controls had higher -dP/dt values relative to untreated controls at 10 cm H₂O and 12.5 cm H₂O of filling pressures. In addition, myo-inositol plus T₃ treated diabetic rats had lower -dP/dt values at filling pressures greater than 15 cm H₂O when compared to myo-inositol treated diabetic rats. Thus it is evident that STZ-treatment resulted in a depressed cardiac function, and
that cardiac functions of diabetic rats improved with myo-inositol treatment alone and also with myo-inositol plus $T_3$ treatment. However, the former treatment was more efficacious than the latter. $T_3$ treatment did not appear to have any effect on cardiac dysfunction of diabetic rats.
TABLE II

EFFECT OF DIFFERENT CONCENTRATIONS OF MYO-INOSITOL TREATMENT ON PLASMA GLUCOSE, PLASMA AND MYOCARDIAL LIPIDS IN 8 WEEK DIABETIC RATS.

<table>
<thead>
<tr>
<th></th>
<th>Untreated diabetic (n=4)</th>
<th>Inositol-treated diabetic Inositol concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 (n=4)</td>
</tr>
<tr>
<td>Plasma glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg/100 ml)</td>
<td>297 ±47</td>
<td>303 ±35</td>
</tr>
<tr>
<td>Plasma triacylglycerols</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg/100 ml)</td>
<td>320 ±14</td>
<td>251 ±17*</td>
</tr>
<tr>
<td>Plasma cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg/100 ml)</td>
<td>81 ±15</td>
<td>77 ±7</td>
</tr>
<tr>
<td>Myocardial triacylglycerols</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(nmol/mg protein)</td>
<td>4.70 ±0.23</td>
<td>4.55 ±0.14</td>
</tr>
<tr>
<td>Myocardial cholesterols</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(nmol/mg protein)</td>
<td>17.1 ±0.3</td>
<td>16.3 ±0.8</td>
</tr>
</tbody>
</table>

Oral myo-inositol treatment at different concentrations (2,3,4,6,8 mg/ml) was initiated 3 days after induction of diabetes and conducted for 8 weeks in inositol-treated diabetic rats. Results are the mean±S.E.M. at sacrifice for number of samples indicated in parentheses; n = number of animals.

*Significantly different from untreated diabetics, p<0.05.
†Significantly different from inositol-treated diabetics at inositol concentrations of 2,6 and 8 mg/ml, p<0.05.
TABLE IIa
EFFECT OF DIFFERENT CONCENTRATIONS OF MYO-INOSITOL ON FLUID INTAKE AND ESTIMATED INOSITOL INTAKE OF 8-WEEK DIABETIC RATS

<table>
<thead>
<tr>
<th>Inositol-treated diabetic inositol concentration (mg/dl)</th>
<th>2 (n=4)</th>
<th>3 (n=4)</th>
<th>4 (n=4)</th>
<th>6 (n=4)</th>
<th>8 (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid intake (ml/day)</td>
<td>150±3</td>
<td>107±2</td>
<td>128±3</td>
<td>171±4</td>
<td>106±2</td>
</tr>
<tr>
<td>Estimated inositol dose (g/kg/day)</td>
<td>1.27</td>
<td>1.40</td>
<td>2.27</td>
<td>4.64</td>
<td>3.58</td>
</tr>
</tbody>
</table>

1. The calculation of "estimated inositol dose" was based on the average fluid intake and final body weight.
2. Results show that inositol dose increased to a certain extent when inositol concentrations increased.
TABLE III

GENERAL FEATURES OF EXPERIMENTAL RATS
USED FOR MYO-INOSITOL STUDY.

<table>
<thead>
<tr>
<th></th>
<th>CON (n=6)</th>
<th>COI (n=6)</th>
<th>DIA (n=5)</th>
<th>DII (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BODY WEIGHT (g)</td>
<td>282 ±10</td>
<td>285 ±9</td>
<td>229 ±6* t</td>
<td>221 ±3* t</td>
</tr>
<tr>
<td>PLASMA GLUCOSE (mg/dl)</td>
<td>123 ±8</td>
<td>133 ±5</td>
<td>390 ±13* t</td>
<td>374 ±20* t</td>
</tr>
<tr>
<td>PLASMA INSULIN (uU/ml)</td>
<td>49 ±4</td>
<td>43 ±5</td>
<td>17 ±0.3* t</td>
<td>16 ±2* t</td>
</tr>
</tbody>
</table>

Myo-inositol (2.5 g/kg/day) was administered orally for 8 weeks to rats 3 days after induction of diabetes with 55 mg/kg i.v. STZ; CON, untreated controls; COI, myo-inositol-treated controls; DIA, untreated diabetics; DII, myo-inositol-treated diabetics; n = number of animals; results are expressed as the mean±S.E.M.

*Significantly different from untreated controls, p<0.05.
†Significantly different from myo-inositol-treated controls, p<0.05.
TABLE IV

FLUID AND MYO-INOSITOL INTAKE OF EXPERIMENTAL RATS
USED FOR MYO-INOSITOL TREATMENT STUDY

<table>
<thead>
<tr>
<th></th>
<th>CON (n=6)</th>
<th>COI (n=6)</th>
<th>DIA (n=5)</th>
<th>DII (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLUID INTAKE</td>
<td>48 ±1</td>
<td>50 ±1</td>
<td>157 ±2*†</td>
<td>161 ±4*†</td>
</tr>
<tr>
<td>(ml/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INOSITOL INTAKE</td>
<td>0 ±0.06</td>
<td>2.50 ±0.06</td>
<td>0</td>
<td>2.49 ±0.05</td>
</tr>
<tr>
<td>(g/kg/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The concentration of myo-inositol in water was adjusted to give the myo-inositol dose at 2.5 g/kg/day. Myo-inositol (2.5 g/kg/day) was given in the drinking water for 8 weeks to rats 3 days after induction of diabetes with 55 mg/kg i.v. STZ; CON, untreated controls; COI, myo-inositol-treated controls; DIA, untreated diabetics; DII, myo-inositol-treated diabetics; n, number of animals; results are expressed as mean±S.E.M.

*Significantly different from untreated controls, p<0.05.
†Significantly different from myo-inositol-treated controls, p<0.05.


TABLE V

THYROID STATUS OF EXPERIMENTAL RATS
USED FOR MYO-INOSITOL STUDY

<table>
<thead>
<tr>
<th></th>
<th>CON (n=6)</th>
<th>COI (n=6)</th>
<th>DIA (n=5)</th>
<th>DII (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLASMA T₃ (ng/ml)</td>
<td>0.457 ±0.043</td>
<td>0.477 ±0.040</td>
<td>0.330 ±0.012*†</td>
<td>0.311 ±0.040*†</td>
</tr>
<tr>
<td>PLASMA T₄ (ug/dl)</td>
<td>3.20 ±0.13</td>
<td>3.73 ±0.38</td>
<td>2.19 ±0.23*†</td>
<td>2.28 ±0.11*†</td>
</tr>
</tbody>
</table>

Myo-inositol (2.5 g/kg/day) was given in the drinking water for 8 weeks to rats 3 days after induction of diabetes with 55 mg/kg i.v. STZ; CON, untreated controls; COI, myo-inositol-treated controls; DIA, untreated diabetics; DII, myo-inositol-treated diabetics; n = number of animals; results are expressed as the mean±S.E.M.

*Significantly different from untreated controls, p<0.05.
†Significantly different from myo-inositol-treated controls, p<0.05.
FIGURE 3

Effect of myo-inositol treatment on plasma triacylglycerols of control and STZ-diabetic rats.

Myo-inositol was given at a dose of 2.5 g/kg/day for 8 weeks. Plasma samples were collected at the time of sacrifice in untreated controls (CON, □, n=6), myo-inositol-treated controls (COI, □ n=6), untreated diabetics (DIA, □, n=5), and myo-inositol-treated diabetics (DII, □, n=6). Measurement of triacylglycerols is as described in Methods. Results shown are the mean±S.E.M.

§Significantly different from the other three groups.
PLASMA TRIACYLGLYCEROLS

CON (N=6)  

DII (N=6)  

COI (N=6)  

DIA (N=5)  

Plasma Triacylglycerols (mg/100 mL)

MYO-INOSONITOL TREATMENT
FIGURE 4

Effect of myo-inositol-treatment on plasma cholesterol of control and STZ-diabetic rats.

Myo-inositol was given at a dose of 2.5 g/kg/day for 8 weeks. Plasma samples were collected at the time of sacrifice in untreated controls (CON, □, n=6), myo-inositol-treated controls (COI, ◻, n=6), untreated diabetics (DIA, ◊, n=5), and myo-inositol-treated diabetics (DII, ○, n=6). Measurement of cholesterol is as described in Methods. Results shown are the mean±S.E.M.

$Significantly different from the other three groups, p<0.05.
PLASMA CHOLESTEROL

CDI (N=8)  
DII (N=6)  
DIA (N=5)

Plasma Cholesterol (mg/100 ml)

0  30  60  90  120

MYO-INOSITOL TREATMENT
FIGURE 5

Effect of myo-inositol treatment on plasma phospholipids of control and STZ-diabetic rats.

Myo-inositol was given at a dose of 2.5 g/kg/day for 8 weeks. Plasma samples were collected at the time of sacrifice in untreated controls (CON, □, n=6), myo-inositol-treated controls (COI, □, n=6), untreated diabetics (DIA, □, n=5), and myo-inositol-treated diabetics (DII, □, n=6). Measurement of triacylglycerols is as described in methods. Results shown are the mean±S.E.M.

$\$Significantly different from the other three groups, p<0.05.
FIGURE 6

Effect of myo-inositol treatment on myocardial triacylglycerols of control and STZ-diabetic rats.

Myo-inositol was given at a dose of 2.5 g/kg/day for 8 weeks. Lipids were extracted from heart homogenates. Measurement of triacylglycerols is as described in Methods. Results shown are the mean±S.E.M.

*Significantly different from untreated controls, p<0.05
†Significantly different from myo-inositol-treated controls, p<0.05.
‡ Significantly different from untreated diabetics, p<0.05.

CON, untreated controls; COI, myo-inositol-treated controls; DIA, untreated diabetics; DII, myo-inositol-treated diabetics.
MYOCARDIAL TRIACYLGLYCEROLS

\[ \text{CON (N=6)} \quad \text{COI (N=6)} \quad \text{DIA (N=5)} \]

\[ \text{N=6} \]

\[ \text{N=6} \]

\[ \text{N=6} \]

\[ \text{nmol TRIACYLGLYCEROLS/mg PROTEIN} \]

\[ \text{MYO-INOSITOL TREATMENT} \]
FIGURE 7

Effect of myo-inositol treatment on myocardial cholesterol of control and STZ-diabetic rats.

Myo-inositol was given a dose of 2.5 g/kg/day for 8 weeks. Lipids were extracted from heart homogenates. Measurement of cholesterol is as described in Methods. Results shown are the mean±S.E.M.

§Significantly different from the other three groups, p<0.05.

CON, untreated controls; COI, myo-inositol-treated controls; DI, untreated diabetics; DII, myo-inositol-treated diabetics.
MYOCARDIAL CHOLESTEROL

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>6</td>
</tr>
<tr>
<td>CDI</td>
<td>8</td>
</tr>
<tr>
<td>DIA</td>
<td>5</td>
</tr>
</tbody>
</table>

**Legend:**
- **CON** (N=6)
- **CDI** (N=8)
- **DIA** (N=5)

**Chart:**
- **X-axis:** MYO-INOSITOL TREATMENT
- **Y-axis:** nmol CHOLESTEROL/mg PROTEIN

- Bars represent different treatments with varying cholesterol levels.
FIGURE 8

Effect of myo-inositol treatment on myocardial phospholipids of control and STZ-diabetic rats.

Myo-inositol was given at a dose of 2.5 g/kg/day for 8 weeks. Lipids were extracted from heart homogenates. Measurement of phospholipids is as described in Methods. Results shown are the mean±S.E.M.

*Significantly different from untreated controls, P<0.05.
†Significantly different from myo-inositol-treated controls, p<0.05.
‡ Significantly different from untreated diabetics, p<0.05.

CON, untreated controls, COI, myo-inositol-treated controls; DIA, untreated diabetics; DII, myo-inositol-treated diabetics.
nmol PHOSPHOLIPIDS/mg PROTEIN

MYOCARDIAL PHOSPHOLIPIDS

MYO-INOSITOL TREATMENT

(CON) (CO1) (DIA)

(N=6) (N=9) (N=5)
Figure 9

Effect of diabetes and myo-inositol treatment on LVDP of isolated perfused working hearts at various filling pressures.

Hearts were isolated from control (CON, ●, n=5), myo-inositol-treated control (COI, □, n=5), diabetic (DIA, △, n=4) and myo-inositol-treated diabetic (DII, ▼, n=5) rats 60 days after the induction of diabetes with 55 mg/kg i.v. STZ. Myo-inositol-treated control and diabetic rats received 2.5 g/kg/day myo-inositol orally commencing 3 days after induction of diabetes. Results are the mean±S.E.M. for number of hearts shown in parentheses.

*Significantly different from untreated controls, p<0.05.
†Significantly different from myo-inositol-treated controls, p<0.05.
‡Significantly different from untreated diabetics, p<0.05.
FIGURE 10

Effect of diabetes and myo-inositol-treatment on +dP/dt of isolated perfused working hearts at various filling pressures.

Hearts were isolated from control (CON, ●, n=5); myo-inositol-treated control (COI, □, n=5); diabetic (DIA, ▲, n=4) and myo-inositol-treated diabetic (DII, ▼, n=5) rats 60 days after the induction of diabetes 55 mg/kg i.v. STZ. Myo-Inositol-treated control and diabetic rats received 2.5 g/kg/day myo-inositol orally commencing 3 days after induction of diabetes. Results are the mean±S.E.M. for number of hearts shown in parentheses.

*Significantly different from untreated controls, p<0.05
†Significantly different from myo-inositol-treated control, p<0.05.
‡Significantly different from untreated diabetics, p<0.05.
FIGURE 11

Effect of diabetes and myo-inositol-treatment on -dP/dt of isolated perfused working hearts at various filling pressures.

Hearts were isolated from control (CON, ●, n=5), myo-inositol-treated control (COI, ■, n=5), diabetic (DIA, ▲, n=4) and myo-inositol-treated diabetic (DII, ▼, n=5) rats 60 days after the induction of diabetes with 55 mg/kg i.v. STZ. Myo-inositol-treated control and diabetic rats received 2.5 g/kg/day myo-inositol orally commencing 3 days after induction of diabetes. Results are the mean±S.E.M. for number of hearts shown in parentheses.

*Significantly different from untreated controls, p<0.05.
†Significantly different from myo-inositol-treated controls, p<0.05
‡Significantly different from untreated diabetics, p<0.05.
§Significantly different from the other three groups, p<0.05.
NEGATIVE $dP/dT$

- CON (N=5)
- COI (N=5)
- DIA (N=4)
- DII (N=5)

**Graph:**
- NEGATIVE $dP/dT$ (mmHg/s) vs. FILLING PRESSURE (cm H$_2$O)
- Data points for each group (CON, COI, DIA, DII) are plotted.
- Error bars indicate variability.
- Statistical significance symbols (e.g., *, †) are shown on the graph.
### TABLE VI

**GENERAL FEATURES OF EXPERIMENTAL RATS USED FOR MYO-INOSITOL (MI) AND TRIIODOTHYRONINE (T₃) TREATMENT STUDY**

<table>
<thead>
<tr>
<th></th>
<th>CON (n=5)</th>
<th>COI (n=4)</th>
<th>COT (n=7)</th>
<th>CIT (n=7)</th>
<th>DIA (n=5)</th>
<th>DII (n=4)</th>
<th>DIT (n=6)</th>
<th>DIIT (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BODY WEIGHT (g)</strong></td>
<td>254 ±13</td>
<td>259 ±11</td>
<td>255 ±8</td>
<td>266 ±6</td>
<td>196 ±16 †</td>
<td>220 ±14* †</td>
<td>218 ±8* †</td>
<td>216 ±6* †</td>
</tr>
<tr>
<td><strong>PLASMA GLUCOSE (mg/dl)</strong></td>
<td>135 ±11</td>
<td>141 ±13</td>
<td>125 ±7</td>
<td>114 ±2</td>
<td>421 ±17 †</td>
<td>411 ±12 †</td>
<td>419 ±4 †</td>
<td>385 ±10 †</td>
</tr>
<tr>
<td><strong>PLASMA INSULIN (uU/ml)</strong></td>
<td>41 ±4</td>
<td>40 ±3</td>
<td>37 ±4</td>
<td>47 ±5</td>
<td>15 ±1 †</td>
<td>16 ±2 †</td>
<td>17 ±1 †</td>
<td>15 ±2 †</td>
</tr>
</tbody>
</table>

Myo-inositol (2.5 g/kday, orally) and/or T₃ (30 ug/kg/day, s.c.) were given for 8 weeks to rats 3 days after induction of diabetes with 55 mg/kg i.v. STZ. The different groups are represented by the following letters: CON, untreated controls; COI, MI-treated controls; COT, T₃-treated controls; CIT, MI + T₃-treated controls; DIA, untreated diabetics; DII, MI-treated diabetics; DIT, T₃-treated diabetics; DIIT, MI + T₃-treated diabetics; n, number of animals. Results are expressed as the mean±S.E.M.

*Significantly different from untreated controls, (p<0.05).
†Significantly different from all controls, (p<0.05).
‡Significantly different from controls of same treatment group (p<0.05).
TABLE VII
FLUID AND MYO-INOSITOL INTAKE OF EXPERIMENTAL RATS
USED FOR MYO-INOSITOL (MI) AND TRIIODOTHYRONINE (T₃) -TREATMENT STUDY.

<table>
<thead>
<tr>
<th></th>
<th>CON (n=5)</th>
<th>COI (n=4)</th>
<th>COT (n=7)</th>
<th>CIT (n=7)</th>
<th>DIA (n=5)</th>
<th>DII (n=4)</th>
<th>DIT (n=6)</th>
<th>DIIT (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLUID INTAKE (ml/day)</td>
<td>44 ±1</td>
<td>37 ±1 *</td>
<td>47 ±1 *</td>
<td>49 ±1 *</td>
<td>170 ±2 *</td>
<td>127 ±2*§</td>
<td>194 ±5*§</td>
<td>186 ±3*§</td>
</tr>
<tr>
<td>INOSITOL INTAKE (g/kg/day)</td>
<td>0 ±0.09</td>
<td>2.54 ±0.09</td>
<td>0 ±0.06</td>
<td>2.53 ±0.06</td>
<td>0 ±0.04</td>
<td>2.51 ±0.04</td>
<td>0 ±0.03</td>
<td>2.52 ±0.03</td>
</tr>
</tbody>
</table>

The concentration of myo-inositol in water was adjusted to give the myo-inositol dose at 2.5 g/kg/day. Myo-inositol (2.5 g/kg/day, orally) and/or T₃ (30 μg/kg/day, s.c.) were given for 8 weeks to rats 3 days after induction of diabetes with 55 mg/kg i.v. STZ. The different groups are represented by the following letters: CON, untreated controls; COI, MI-treated controls; COT, T₃-treated controls; CIT, MI + T₃-treated controls; DIA, untreated diabetics; DII, MI-treated diabetics; DIT, T₃-treated diabetics; DIIT, MI + T₃-treated diabetics; n, number of animals. Results are expressed as the mean±S.E.M.

*Significantly different from untreated controls (p<0.05).
†Significantly different from all controls (p<0.05).
§Significantly different from controls of the same treatment group (p<0.05).
§§Significantly different from untreated diabetics (p<0.05).
TABLE VIII

THYROID STATUS OF EXPERIMENTAL RATS USED FOR MYO-INOSITOL (MI) AND TRIIODOTHYRONINE (T₃) -TREATMENT STUDY.

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>COI</th>
<th>COT</th>
<th>CIT</th>
<th>DIA</th>
<th>DII</th>
<th>DIT</th>
<th>DIIT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=5)</td>
<td>(n=4)</td>
<td>(n=7)</td>
<td>(n=7)</td>
<td>(n=5)</td>
<td>(n=4)</td>
<td>(n=6)</td>
<td>(n=7)</td>
</tr>
<tr>
<td>PLASMA T₃ (ng/ml)</td>
<td>0.483</td>
<td>0.588</td>
<td>0.734</td>
<td>0.676</td>
<td>0.340</td>
<td>0.355</td>
<td>0.506</td>
<td>0.510</td>
</tr>
<tr>
<td>±0.023 ±0.046 ±0.042 ±0.035 ±0.015† ±0.016† ±0.023 ±0.024</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLASMA T4 (nmol/l)</td>
<td>53</td>
<td>50</td>
<td>16</td>
<td>22</td>
<td>39</td>
<td>37</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>±4 ±4 ±1* ±1* ±3† ±1† ±3§ ±1§</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Myo-inositol (2.5 g/kg/day, orally) and/or T₃ (30 ug/kg/day, s.c.) were given for 8 weeks to rats 3 days after induction of diabetes with 55 mg/kg i.v. STZ. The different groups are represented by the following letters: CON, untreated controls; COI, MI-treated controls; COT, T₃-treated controls; CIT, MI + T₃-treated controls; DIA, untreated diabetics; DII, MI-treated diabetics; DIT, T₃-treated diabetics; DIIT, MI + T₃-treated diabetics; n, number of animals. Results are expressed as the mean±S.E.M.

*Significantly different from untreated controls (p<0.05).
†Significantly different from all controls (p<0.05).
§Significantly different from controls of the same treatment group (p<0.05).
§§Significantly different from untreated diabetics (p<0.05).
Levels of triacylglycerols in plasma obtained from control and diabetic rats treated with myo-inositol and T₃.

Plasma samples were collected at time of sacrifice in untreated controls (CON, □, n=5), myo-inositol-treated controls (COI, ■, n=4), T₃-treated controls (COT, ▪, n=7), myo-inositol plus T₃-treated controls (CIT, ▼, n=7), untreated diabetics (DIA, □, n=5), myo-inositol treated diabetics (DII, ▪, n=4), T₃-treated diabetics (DIT, ▼, n=6), and myo-inositol-plus-T₃-treated diabetics (DIIT, ▼, n=7). Measurement of triacylglycerols is as described in Methods. Results shown are the mean±S.E.M.

*Significantly different from untreated controls, p<0.05.
†Significantly different from all controls, p<0.05.
‡Significantly different from controls of same treatment group, p<0.05.
§Significantly different from untreated diabetics, p<0.05.
PLASMA TRIACYLGLYCEROLS

CON (N=5)
CIT (N=7)
DIT (N=6)
COI (N=4)
DIA (N=5)
DII (N=7)
COT (N=7)
DIIIT (N=4)

Plasma Triacylglycerols (mg/100 ml)

0 130 260 390 520 650 780

MYO-INOSITOL AND T₃ TREATMENT
Levels of cholesterol in plasma obtained from control and diabetic rats treated with myo-inositol and T₃.

Plasma samples were collected at the time of sacrifice in untreated controls (CON, □, n=5), myo-inositol-treated controls (COI, ¶, n=4), T₃-treated controls (COT, ■, n=7), myo-inositol plus T₃-treated controls (CIT, ¶, n=7), untreated diabetics (DIA, □, n=5), myo-inositol-treated diabetics (DII, ¶, n=4), T₃-treated diabetics (DIT, ¶, n=6) and myo-inositol plus T₃-treated diabetics (DIIT, ¶, n=7). Measurement of cholesterol is as described in Methods. Results shown are the mean±S.E.M.

*Significantly different from untreated controls, p<0.05.
†Significantly different from all controls, p<0.05.
‡Significantly different from controls of same treatment group, p<0.05.
§Significantly different from untreated diabetics, p<0.05.
FIGURE 14

Levels of phospholipids in plasma obtained from control and diabetic rats treated with myo-inositol and T3.

Plasma samples were collected at the time of sacrifice in untreated controls (CON, □, n=5), myo-inositol-treated controls (COI, ▮, n=4), T3-treated controls (COT, ▮, n=7), myo-inositol plus T3 treated controls (CIT, ▮, n=7), untreated diabetics (DIA, □, n=5), myo-inositol-treated diabetics (DII, ▮, n=4), T3-treated diabetics (DIT, ▮, n=6) and myo-inositol plus T3-treated diabetics (DIIT, ▮, n=7). Measurement of phospholipids is as described in Methods. Results are shown as the mean±S.E.M.

*Significantly different from untreated controls, p<0.05.
†Significantly different from all controls, p<0.05.
‡Significantly different from controls of same treatment group, p<0.05.
§Significantly different from untreated diabetics, p<0.05.
FIGURE 15

Measurement of myocardial triacylglycerol levels in control and diabetic rats administered myo-inositol and T₃.

Lipids were extracted from heart homogenates. Results are shown as the mean±S.E.M.; CON, untreated controls; COI, MI-treated controls; COT, T₃-treated controls; CIT, MI plus T₃-treated controls; DIA, untreated diabetics; DII, MI-treated diabetics; DIT, T₃-treated diabetics; DIIT, MI plus T₃-treated diabetics.

*Significantly different from untreated controls, p<0.05.
†Significantly different from all controls, p<0.05.
‡Significantly different from controls of the same treatment group, p<0.05.
§Significantly different from untreated diabetics, p<0.05.
MYOCARDIAL TRIACYLGLYCEROLS

CON (N=5)  
CIT (N=7)  
DIT (N=6)

COI (N=4)  
DIA (N=5)  
DIIT (N=7)

COT (N=7)  
DII (N=4)

nmol TRIACYLGLYCEROLS/mg PROTEIN

MYO-INOSITOL AND T3 TREATMENT
FIGURE 16

Measurement of myocardial cholesterol levels in control and diabetic rats administered myo-inositol and T₃.

Lipids were extracted from heart homogenates. Results shown are the mean±S.E.M.; CON, untreated controls; COI, MI-treated controls; COT, T₃-treated controls; CIT, MI plus T₃-treated controls; DIA, untreated diabetics; DII, MI-treated diabetics; DIT, T₃-treated diabetics; DIIT, MI plus T₃-treated diabetics.

*Significantly different from untreated controls, p<0.05.
†Significantly different from all controls, p<0.05.
‡Significantly different from controls of same treatment group, p<0.05.
§Significantly different from untreated diabetics, p<0.05.
MYOCARDIAL CHOLESTEROL

MYO-INOSITOL AND T₃ TREATMENT
FIGURE 17

Measurement of myocardial phospholipid levels in control and diabetic rats administered myo-inositol and T₃.

Lipids were extracted from heart homogenates. Results shown are the mean±S.E.M.; CON, untreated controls; COI, MI-treated controls; COT, T₃-treated controls; CIT, MI plus T₃-treated controls; DIA, untreated diabetics; DII, MI-treated diabetics; DIT, T₃-treated diabetics; DIIT, MI plus T₃-treated diabetics.

*Significantly different from untreated controls, p<0.05
†Significantly different from all controls, p<0.05
‡Significantly different from controls of same treatment group, p<0.05
§Significantly different from untreated diabetics, p<0.05.
MYOCARDIAL PHOSPHOLIPIDS

CON (N=5)  COI (N=4)  COT (N=7)
CIT (N=7)  DIA (N=5)  DII (N=4)
DIT (N=6)  DIIT (N=7)

nmol PHOSPHOLIPIDS/mg PROTEIN

MYO-INOSITOL AND T₃ TREATMENT
FIGURE 18

Effect of 8 weeks of myo-inositol and T₃ treatment in diabetic rats on diabetes-induced depression of LVDP.

Results shown are the mean±S.E.M.; CON, untreated controls; COI, MI-treated controls; COT, T₃-treated controls; CIT, MI plus T₃-treated controls; DIA, untreated diabetics; DII, MI-treated diabetics; DIT, T₃-treated diabetics; DIIT, MI plus T₃ treated diabetics.

*Significantly different from untreated controls, p<0.05.
†Significantly different from all controls, p<0.05.
‡Significantly different from controls of the same treatment group, p<0.05.
§Significantly different from untreated diabetics, p<0.05.
¶Significantly different from myo-inositol-treated diabetics, p<0.05.
LVDP

(○) CON (N=5)
(□) COI (N=4)
(△) COT (N=7)
(▼) CIT (N=7)
(●) DIA (N=5)
(■) DII (N=4)
(▲) DIT (N=6)
(▼) DIIT (N=7)

LVDP (mmHg)

FILLING PRESSURE (cm H₂O)
FIGURE 19

Effect of 8 weeks of myo-inositol and T₃ treatment in diabetic rats on diabetes-induced depression of +dP/dt.

Results are shown as the mean±S.E.M.; CON, untreated controls; COI, MI-treated controls; COT, T₃-treated controls; CIT, MI plus T₃-treated controls; DIA, untreated diabetics; DII, MI-treated diabetics; DIT, T₃-treated diabetics; DIIT, MI plus T₃-treated diabetics.

*Significantly different from untreated controls, p<0.05.
†Significantly different from all controls, p<0.05.
‡Significantly different from controls of same treatment group, p<0.05.
§Significantly different from untreated diabetics, p<0.05.
¶Significantly, different from myo-inositol-treated diabetics.
POSITIVE $\frac{dP}{dT}$

- $\bullet$ CCN ($N=5$)
- $\circ$ COI ($N=4$)
- $\triangle$ COT ($N=7$)
- $\triangledown$ CIT ($N=7$)
- $\bullet$ DIA ($N=5$)
- $\mathbf{■}$ DII ($N=4$)
- $\mathbf{▲}$ DIT ($N=6$)
- $\mathbf{▼}$ DII T ($N=7$)

**TILLING PRESSURE** (cm H$_2$O)

**POSITIVE** $\frac{dP}{dT}$ (mm Hg/s)
FIGURE 20

Effect of 8 weeks of myo-inositol and $T_3$ treatment in diabetic rats on diabetes-induced depression of $-\frac{dP}{dt}$.

Results are shown as the mean±S.E.M.; CON, untreated controls; COI, MI-treated controls; COT, $T_3$-treated controls; CIT, MI plus $T_3$-treated controls; DIA, untreated diabetics; DII, MI-treated diabetics; DIT, $T_3$-treated diabetics; DIIT, MI plus $T_3$-treated diabetics.

*Significantly different from untreated controls, p<0.05.
†Significantly different from controls, p<0.05.
‡Significantly different from controls of the same treatment group, p<0.05.
§Significantly different from untreated diabetics, p<0.05.
¶Significantly different from myo-inositol-treated diabetics, p<0.05.
NEGATIVE $dP/dT$

- (○) CON (N=5)
- (□) CDI (N=4)
- (△) COT (N=7)
- (▼) CIT (N=7)
- (●) DIA (N=5)
- (■) DII (N=4)
- (▲) DIT (N=6)
- (▼) DIIIT (N=7)

FILLING PRESSURE (cm H₂O)

NEGATIVE $dP/dT$ (mm Hg/s)
The present study was designed to investigate the effect of myo-inositol and/or T₃ treatment on cardiac dysfunction and elevated myocardial lipid levels in STZ-diabetic rats.

Diabetes was induced by the intravenous injection of STZ (55 mg/kg) which directly destroys pancreatic beta cells. The diabetic state was characterized by hyperglycemia and loss of body weight as early as 3 days after STZ injection. Absolute or relative deficiency of insulin can lead to a marked decrease in the cellular rate of glucose uptake, resulting in elevated levels of blood glucose. The decrease in body weight at the time of sacrifice occurred in spite of increased fluid and food intake and was probably due to dehydration and protein and fat catabolism during diabetes (Oakley, 1968).

In our study, the choice of an 8 week study period was based on previous work done in our laboratory as well as the work of others. Fein et al. (1980) reported that changes in papillary muscle mechanics of STZ-diabetic rats were seen five weeks after the onset of diabetes. A study by Penpargkul et al. (1980) demonstrated that eight weeks after STZ treatment diabetic rats exhibited depressed cardiac function. Similarly, Vadlamudi et al. (1982) found that the onset of depression of cardiac performance of STZ diabetic rats was between 30 days and 3 months of diabetes. In a study by Tahilian et al. (1983), STZ treated rats exhibited the depression 6 weeks after the induction of diabetes. Thus it appears
that myocardial changes occur 4 to 8 weeks after the induction of diabetes. Results from our study showed that hearts from 8 week diabetic rats exhibited a depression in cardiac function compared to controls which is in general agreement with the above observations.

An association between hyperlipidemia and diabetic ketoacidosis was first demonstrated by Bloor (1916). Since then, elevated blood lipids have been widely reported among patients with poorly controlled diabetes mellitus. It has been estimated that the frequency of elevated plasma lipid levels in diabetic patients is between 20 and 90% (Billmoría et al., 1976; Simpson et al., 1979) depending on the degree of diabetic control and the type of diabetes. The most commonly and markedly elevated fasting plasma lipid level is triacylglycerol (New et al. 1963; Nikkila, 1974) associated with a less pronounced increase in plasma cholesterol and phospholipid in the diabetic population (Adlersberg and Eisler, 1959; Albrink et al. 1963; Medalie et al., 1974). Lipid alterations have also been reported in chemically-induced animal models. In non-human primates during insulin deprivation, plasma triacylglycerols increase first and primarily, followed by plasma cholesterol and phospholipids (Howard, 1975). Similarly, an elevation of plasma free fatty acid, triacylglycerols, cholesterol and phospholipids were shown in STZ diabetic rats (Rodrigues et al. 1986). Elevation of plasma lipids indicates either the defective removal or overproduction or both of one or more lipoproteins. Because of the role of insulin in both production and removal of triacylglycerol-rich lipoproteins, insulin deficiency may be the major cause of lipid disorders of diabetes. Evidence strongly suggests that there
is a defect in the removal of triacylglycerol-rich lipoproteins in insulin-deficient animals and man that is restored to normal by the administration of insulin (Nikkila et al., 1977). Furthermore, lipoprotein lipase (LPL), which is the enzyme responsible for the removal of triacylglycerol-rich lipoprotein, has been demonstrated to fall after insulin withdrawal in insulin-dependent diabetic animals (Elkeles and Hambley, 1977) and man (Bagdade et al. 1968) and is low in poorly controlled diabetic patients (Nikkila et al. 1977). Administration of insulin to patients was found to increase LPL activity above normal (Nikkila et al. 1977).

Overproduction of lipoproteins is another biochemical abnormality involved in increased plasma lipids in diabetes. Evidence suggests that increased very low density lipoproteins (VLDL) production by the liver is more commonly shown in patients with insulin resistance (Kissebah et al., 1974). In addition, it is suggested that during insulin resistance insulin is ineffective in suppressing lipolysis in adipose tissue, which may also account for the elevation in plasma cholesterol and triacylglycerols (Goldberg, 1981).

Clinical evidence has shown that thyroid hormones also play a role in plasma lipoprotein and cholesterol metabolism. Hypercholesterolaemia is almost a clinical prerequisite of severe hypothyroidism. Studies in cultured cells (Chait et al. 1979) and the intact organism (Thompson et al. 1981) indicate that T₃ or thyroxine deficiency is associated with defective expression of low density lipoprotein (LDL) receptor activity, leading to abnormal catabolism of lipoprotein and cholesterol. Replacement therapy, on the other hand, increased receptor activity, promoted LDL catabolism
and therefore reduced cholesterol levels in the blood. Diabetes is known to result in a state of hypothyroidism (Pittman et al. 1979). It is thus possible that elevated plasma cholesterol levels could be a result of diabetes-induced hypothyroidism.

Our results regarding the effects of STZ diabetes on plasma lipid levels suggest that elevated levels of plasma lipid could result from either low plasma insulin levels and an associated decreased removal of lipoproteins, or diabetes-induced hypothyroidism.

The involvement of elevated levels of circulating lipids in the development of vascular and heart diseases has been suggested in both diabetic man and animals. Pomeranze and Kunkel (1950) showed a general correlation between arteriosclerosis and high total lipid, high cholesterol or reduced phospholipid/cholesterol ratio. Adlersberg and Eisler (1959) suggested that elevation of plasma fatty acid and triacylglycerol levels were related to the presence of vascular complications in diabetic patients. Similarly, studies by New et al. (1963) showed a relationship between complications (such as myocardial infarction and peripheral vascular disease) and elevated plasma triacylglycerol concentration in diabetics aged 31 to 50. Also it is of interest that non-diabetics in this middle age group were found to have elevated triacylglycerol levels which have been correlated with the early development of ischemic heart disease (Antonis and Bersohn, 1960; Carlson, 1960). One line of evidence in the animal studies that implicates elevated levels of circulating fats in the production of diabetic cardiomyopathy is that insulin treatment in diabetic animals not only causes
a glucose lowering effect but also decreases the levels of circulating fats which is accompanied by a recovery of cardiac function (Mochizuki et al., 1984). In a study by Rodrigues and McNeill (1986), Wistar and Wistar-Kyoto (WKY) rats were injected with identical doses of STZ and both strains of rats exhibited hyperglycemia and hypoinsulinemia. However, only the Wistar rats exhibited hyperlipidemia and depressed myocardial function, while neither hyperlipidemia nor cardiac dysfunction was seen in rats of the WKY strain. In another study, hydralazine treatment normalized circulating lipid levels as well as the function of hearts in diabetic rats (Rodrigues et al., 1986). However, a combination of thyroid hormone and methylpalmoxirrate which was able to partially improve cardiac function did not have any effect on elevated levels of circulating lipids in diabetic rats (Tahiliani and McNeill, 1985). Furthermore, Heyliger et al. (1986) showed that choline and/or methionine improved the cardiac performance of diabetic rats which was accompanied by a reduction in both plasma and myocardial lipids. These results suggest that circulating lipids are not the only determinant of cardiac function in diabetes and myocardial lipids may be more directly related to the cardiac function in diabetes.

Myocardial lipid levels in diabetic rats were also measured in our study. Our results demonstrated significant elevations of myocardial lipids in STZ-diabetic rats. A similar lipid accumulation has also been reported by other investigators. Regan et al. (1973), Shipp et al. (1973) and Murthy and Shipp (1977) showed that there was an abnormal triacylglycerol buildup in the diabetic myocardium. In 1986, Heyliger et al. demonstrated an
accumulation of both triacylglycerol and cholesterol in the myocardium in STZ-diabetic rats. Several factors could be involved in this myocardial lipid buildup. Accumulation of triacylglycerol may reside in a defect in the lipase system, which may then lead to a decreased removal of triacylglycerol from the myocardium (Atkin and Meng, 1972). A diminished uptake of chylomicron triacylglycerol has been observed in diabetic rat hearts (Kreisberg, 1966). Evidence of reduced lipoprotein lipase activity supplied by Atkin and Meng (1972) also supports the view that extracellular triacylglycerol does not contribute to the cardiac lipid buildup. Increased plasma FFA levels could also be involved in the myocardial lipid accumulation. In fact both the uptake and esterification of FFA are increased when the perfusate FFA concentration is increased (Opie 1969; Neely and Morgan, 1974). Furthermore, myocardial triacylglycerol content is known to be increased in diabetes in which plasma FFA concentrations are increased (Frederickson and Gordon, 1958; Shipp et al., 1973). However, Regan et al. (1973) failed to observe a close correlation between the increase in plasma FFA and the myocardial buildup of triacylglycerol. On the other hand, studies suggest that increased uptake of plasma FFA could inhibit cardiac triacylglycerol mobilization, which may also contribute to triacylglycerol accumulation in the myocardium of diabetics (Paulson and Crass, 1982; Murthy et al., 1983). In addition it is possible that increased synthesis of triacylglycerol could account for triacylglycerol accumulation in the myocardium of diabetics. However, increased biosynthesis of triacylglycerol is shown to be a factor in its buildup in the myocardium
only in ketotic diabetic rats (Murthy and Shipp, 1977). There is no evidence for the increased myocardial triacylglycerol or fatty acid synthesis in nonketotic diabetic animals (Whereat and Orishimo, 1969). Thus, it seems that impaired triacylglycerol lipase activity and/or inhibited mobilization of cardiac triacylglycerol may play a role in the myocardial triacylglycerol buildup in diabetes.

Myocardial cholesterol buildup could result from either increased synthesis or increased uptake, or both. Increased uptake and associated accumulation of cholesterol in the myocardial cells can downregulate de novo endogenous cholesterol synthesis by inhibiting HMG CoA reductase, the rate-limiting enzyme in cholesterol synthesis. Suppression of this enzyme leaves the cell dependent on external cholesterol (Brown and Goldstein, 1984). Furthermore, the incoming cholesterol promotes the storage of this lipid in the cell by activating the enzyme acyl-CoA: cholesterol acyltransferase (ACAT). In the diabetic state, plasma cholesterol is elevated which may result in increased uptake of this lipid by the heart and accumulation of this lipid in the myocardium. Hence, increased uptake of cholesterol by the heart, rather than increased de novo synthesis in the heart, could be mainly contributed to the increased myocardial cholesterol content.

There is increasing evidence that cardiac function can be affected by alteration in the lipid levels of the myocardium. Henderson et al. (1970) demonstrated that FFA perfusion depressed myocardial contractility in rat hearts subjected to hypoxia. Regan et al. (1973) showed that cardiac dysfunction in chronically diabetic dogs was accompanied by triacylglycerol
increases in cardiac cells. In addition, Peterson et al. (1979) presented evidence that myocardial contractility in papillary muscles was lowered in atherosclerotic rabbits. From our studies, it also appears that changes in the lipid levels of the myocardium are correlated with the depressed performance in the diabetic hearts.

Several mechanisms have been postulated for the depressed cardiac function as a result of myocardial lipid levels. The increase in triacylglycerol and cholesterol in the myocardium may be involved in the left ventricular stiffness of the diabetic heart (Regan et al., 1973). Accumulations of more cholesterol than phospholipid in the cell could increase cholesterol/phospholipid ratio, resulting in a decrease in membrane fluidity. The changes in membrane fluidity may further alter the microenvironment and affect the integrity of both the cellular and subcellular membranes (Pezzoli et al., 1983). The alterations in the integrity of the myocardial membranes could depress the activity of various myocardial enzymes and other transporting systems that play important roles in the normal contraction of the heart on a beat to beat basis. Depressed Na\textsuperscript{+}-K\textsuperscript{+} ATPase (Karli et al. 1979) and Ca\textsuperscript{2+}-Mg\textsuperscript{2+}-dependent ATPase (Panagia et al. 1982) activities in the rat heart SL have been reported. In addition it has been suggested that defective SR calcium transport is related to diabetes-induced metabolic derangements in lipids and concomitant membrane alterations (Ganguly et al. 1983).

It is also known that myocardial lipid buildup can increase cardiac lipid metabolism. The resulting elevated levels of LCAC in the SR preparations derived from chronically diabetic rats could inhibit membrane
transport proteins including SR calcium ATPase (Lopaschuk et al. 1983). Increased cardiac lipid metabolism could also increase cardiac oxygen demand which may lead to anoxia. In diabetes, glycolysis is inhibited by increased FFA and the only substrate available for energy is FFA. FFA cannot be utilized for energy in anoxia and may accumulate intracellularly as triacylglycerol or FFA which may lead to decreased energy supply and decreased oxygen supply in the diabetic myocardium (Opie, 1972).

The present study aimed at examining the effect of myo-inositol alone or in combination with T3 in STZ diabetic rats. Daily oral treatment with myo-inositol improved cardial function in STZ diabetic rats. Furthermore, STZ diabetes elevated plasma and myocardial lipid levels. Our results showed that myo-inositol treatment is effective in decreasing these levels in STZ diabetic animals.

Treatment with STZ resulted in a diabetic state characterized by hyperglycemia, hypoinsulinemia, loss of body weight and increased fluid intake. Similar alterations were also reported by other investigators (Vadlamudi et al. 1982; Rodrigues et al., 1986). Myo-inositol treatment had no effect on these parameters indicating myo-inositol could not alter the diabetic state of rats.

The pronounced effect of myo-inositol in lowering plasma and myocardial triacylglycerol, cholesterol and phospholipids in the STZ diabetic rats are, in general, consistent with previous reports. Gavin and McHenry in 1941 first indicated that myo-inositol could serve as a lipotropic factor. They fed a beef liver fraction to rats which caused the production of acutely
fatty livers containing large amounts of cholesterol. However, when myo-inositol was given at the same time, the development of fatty liver and the accumulation of cholesterol in the liver were prevented. In 1951 Dotti showed that myo-inositol inhibited the expected rise in plasma cholesterol and phospholipid levels in rabbits on high cholesterol diets. Furthermore, Hayashi (1974) observed an accumulation of triacylglycerol and cholesterol in the liver and elevated levels of plasma FFA in rats fed a myo-inositol deficient diet, which was corrected by supplementing the diet with myo-inositol.

The mechanisms by which myo-inositol decreased plasma lipid levels of the diabetic rats is a matter of speculation. Myo-inositol treated diabetic rats, although they still had low plasma insulin, did not have the elevated plasma triacylglycerols, cholesterol and phospholipid seen in the untreated diabetic group. It thus seems that the action of myo-inositol on plasma lipids of diabetic rats may be independent of insulin. Myo-inositol could have induced its effect on adipose tissue lipolysis through the sympathetic nerve system. Hayashi et al. (1974) observed an increased mobilization of fatty acid from adipose tissue to the liver in the inositol-deficient rats. Subsequent work by these investigators (1978) suggested that the increased lipolysis associated with an inositol-deficient state may be mediated by an excitation of the sympathetic nerve terminals innervating in the adipose tissues and the resulting activation of hormone-sensitive lipase. Hence, it is possible that pretreatment with myo-inositol could inhibit lipolysis in adipose tissue by depressing sympathetic nerve terminals, which may then decrease the plasma lipid levels of the diabetic rats.
The myocardial lipid-lowering effect of myo-inositol in these diabetic animals is still not clear. However, myo-inositol is involved in the synthesis of phosphatidylinositol (Paulus and Kennedy, 1960), which further increases the synthesis and secretion of lipoproteins in the liver (Hasan et al., 1970; Hasan et al., 1971). A depression in the levels of total plasma lipoprotein and plasma PI in inositol-deprived dams during lactation has been observed (Burton and Wells, 1971). Thus, myo-inositol supplementation could have enhanced the removal of cholesterol from the myocardium by increasing the levels of lipoproteins in these animals. Triacylglycerol also decreased in the myocardium in these myo-inositol treated rats. This could have been achieved by a decrease in plasma FFA as pointed out earlier.

The effect of myo-inositol on myocardial lipid levels could also be attributed to decreased synthesis of lipids in the myocardium. In fact, increased de novo synthesis of lipogenetic enzymes in the liver during myo-inositol deficiency has been observed (Beach and Flick, 1982). It could be speculated that myo-inositol treatment may decrease the synthesis of lipogenic enzymes in the myocardium. Synthesis of lipogenic enzymes may also be depressed in the liver and adipose tissues. Thus, less lipids would be available for the uptake of the myocardium.

Since elevated myocardial lipid level has been suggested to be one of the important contributors in the development of cardiac dysfunction in diabetic animals, myo-inositol may improve cardiac performance of diabetic rats by lowering myocardial lipid levels, with a concomitant decrease in left ventricular stiffness, decrease in LCAC level and cardiac oxygen demand, and an improvement in myocardial membrane integrity and
subcellular activities. These possible effects of myo-inositol on the cardiac performances of diabetic rats requires further investigation.

In addition, improvements in other cellular defects of the diabetic state by myo-inositol treatment could also be involved in the improved cardiac function of the treated diabetic animals. Myo-inositol is involved in the synthesis of phosphatidyl-inositol which is an integral part of the membrane structure and has important biochemical functions at the membrane level including the mediation of cellular responses to external stimuli, nerve transmission, and the regulation of enzyme activity through specific interactions with various proteins (Holub, 1982). Thus, myo-inositol supplementation may enhance the integrity of the membrane structure of myocardial cells associated with an improvement of biochemical function of the membrane. Myo-Inositol is also involved in the synthesis of inositol trisphosphate (IP$_3$) under muscarinic and alpha-adrenergic receptor stimulation (Brown and Jones, 1986). IP$_3$ has been shown by some investigators (Hirata et al., 1984; Fabiato, 1986; Nosek et al., 1986) to be able to release calcium from cardiac SR, although this has been a subject of controversy. Release of calcium from the SR may then lead to excitation-contraction coupling in cardiac cells. Hence, myo-inositol may improve cardiac performance by releasing calcium from the SR through IP$_3$.

Myo-inositol metabolism has been reported to be altered in diabetic patients (Clements and Reynertson, 1977) and STZ diabetic rats (Goldfarb et al., 1986). An increased renal excretion of myo-inositol (Vohl, 1958; Daughaday et al., 1953) and an intracellular deficiency of myo-inositol (Clements and Reynertson, 1977) have been well documented. Thus it is
possible that supplementation with myo-inositol could have simply improved the myo-inositol status of the diabetic animals, which subsequently restored heart function.

Our study showed that myo-inositol treatment alone partially improved cardiac function and decreased myocardial and plasma lipid levels, while hypothyroidism associated with the diabetes state was still present. Hypothyroidism is known to slow relaxation and to depress the force-velocity relation in papillary muscles (Buccino et al., 1967). Calcium transport by the SR has also been shown to be depressed in the hypothyroid state (Suko, 1973). On the other hand, the increase in calcium accumulating ability of the SR has been observed in hyperthyroid animals (Suko, 1971; Limas, 1978). In addition, greater contractility has been shown in papillary muscles from hearts of guinea pigs treated with thyroxine (Goodkind et al., 1974). Furthermore, Dillman (1982) studied the effect of thyroid hormone replacement therapy on diabetes-induced depression of myosin ATPase. T3 treatment at a pharmacological dose (30 ug/kg·day) successfully prevented diabetes-induced hypothyroidism and normalized cardiac myosin ATPase activity. The decrease in heart rate occurring as a result of diabetes is also correctable by thyroid hormone treatment (Goyal and McNeill, unpublished results). Thus it is possible that cardiac dysfunction seen in diabetics could be due to hypothyroidism associated with the disease.

Therefore, in the third study, both myo-inositol and T3 were administered to the diabetic rats. We wanted to see if the combination treatment could further improve cardiac function in diabetic animals.
As with myo-inositol treatment alone, T₃ alone or in combination with myo-inositol could not alter STZ-induced diabetic state in rats, which was characterized by a loss of body weight and hyperglycemia with corresponding hypoinsulinemia. In addition, untreated diabetic rats had significantly higher fluid intake, which was further increased with T₃ alone or in combination with myo-inositol. The further increase in fluid intake with T₃ treatment may be attributed to the high metabolic state associated with T₃ administration. It is well established that the oxygen consumption and metabolic rate are increased by T₄ and T₃ (Ganong, 1985). Therefore endogenous protein and fat stores are catabolized, which can result in diuresis. Thus increased fluid intake by T₃ treatment may be due to diuresis and a high metabolic state.

When plasma and myocardial lipid levels of the diabetic rats were examined, the lipid levels were found to be unchanged or even increased with T₃ and MI plus T₃ treatment. Cardiac function was depressed in untreated diabetic rats and T₃ treatment alone did not appear to have any effect on cardiac dysfunction. There was, however, some improvement in heart function in the groups treated with both myo-inositol and T₃, but the improvement was not as pronounced as with myo-inositol treatment alone.

The failure of T₃ to improve heart function shown in our study was not unexpected and was consistent with previous work. Fein et al. (1981) observed that there was a dissociation between papillary muscle mechanics and thyroid hormone levels. Furthermore, Tahiliani and McNeill (1984) were
unable to improve cardiac function of diabetic hearts by treating the diabetic rats with thyroid hormone. On the other hand, previous reports have indicated that treating normal (euthyroid) animals with thyroid hormone did result in enhanced cardiac function as compared with untreated euthyroid animals in at least some species (Taylor, 1970; Brooks et al., 1981). In our study, a dose of 30 ug/kg^-1/day^-1 of T_3 was administered to diabetic rats as T_3 treatment at this dose has been previously shown to prevent diabetes-induced hypothyroidism and normalize cardiac myosin ATPase activity. However, cardiac function of diabetic rats remain depressed. These observations confirm the suggestions made by Tahiliani and McNeill (1984) that cardiac dysfunction of diabetic animals does not seem to result from hypothyroidism and decreased myosin ATPase activity.

A less pronounced improvement of cardiac function of diabetic animals was observed with myo-inositol plus T_3 treatment compared to myo-inositol alone. This could be related to the calorigenic and diabetogenic effects of thyroid hormones. As discussed above, a major effect of thyroid hormones is to stimulate oxygen consumption and increase metabolic rate (calorigenic effect). Thus, it is possible that when the metabolic rate is increased, the need for myo-inositol is increased. Myo-inositol supplementation at the same dose as when myo-inositol was given alone may not be sufficient for the diabetic animals who received T_3 at the same time. Thyroid hormones have also been shown to have a diabetogenic effect. Thyrotoxicosis was reported to aggravate clinical diabetes and thyroid diabetes can be
produced in animals with decreased pancreatic reserve (Ganong, 1985). Several mechanisms have been postulated for this diabetogenic effect of thyroid hormones. Thyroid hormones may increase absorption of glucose from the intestine (Ganong, 1985). The hormones may also cause some degree of hepatic glycogen depletion probably by potentiating the effects of catcholamines. Glycogen-depleted liver cells are easily damaged. When the liver is damaged, it takes up less of the absorbed glucose. In addition, thyroid hormones may enhance glycolysis and gluconeogenesis (Guyton, 1986). Furthermore, thyroid hormones may accelerate the degradation of insulin. All these actions have a hyperglycemic effect and, when the pancreatic reserve is already low, may make experimental diabetes worse. Therefore, even though myo-inositol was supplemented, the combination of both could not further improve heart function of diabetic animals.

$T_3$ alone or in combination with myo-inositol did not have any effect on or even further increase the lipid levels of diabetic rats compared to untreated diabetic rats. Several mechanisms may be involved in the elevation of lipid levels of plasma and myocardium of diabetic rats treated with $T_3$. Thyroid hormones may stimulate cholesterol synthesis in the liver (Goodman and Middlesworth, 1980). Thyroid hormones may also increase the synthesis and mobilization of FFA from adipose tissue. There is evidence that in hypothyroid rats the synthesis of long-chain fatty acids from glucose and other carbohydrate precursors is diminished. Similarly, oxidation of long-chain fatty acids is reduced (Bray and Goodman, 1968). Thus, administration of $T_3$ may increase myocardial lipids by increasing synthesis and release of FFA from adipose tissue and increasing the concentrations of FFA in plasma.
The present study shows that myo-inositol treatment influenced lipid metabolism and improved cardiac performance in STZ diabetic rats. While it appears that control of lipid levels is an important factor in the improvement of cardiac function of diabetic animals, it should be noted that the myo-inositol-plus-\(T_3\) treatment did not affect the elevated lipid levels of diabetic rats even though the heart function of these diabetic animals was partially improved. It will nevertheless be of interest in future studies to investigate the mechanism of action of myo-inositol in lipid metabolism. Since myo-inositol metabolism is altered in diabetes and myo-inositol and its derivatives have been suggested to act as second messengers in mediating a positive inotropic response in the heart, myo-inositol supplementation could be of great importance in regulating myocardial contractility at the cellular and subcellular levels in diabetic animals and man.
CONCLUSIONS

1. STZ-induced 8-week diabetic rats exhibited cardiac dysfunction as assessed by a depression of the LVDP, rate of pressure rise and rate of pressure decline in the isolated working heart.

2. Paralleling the depression of cardiac function, an elevation in plasma and myocardial triacylglycerols, cholesterol and phospholipids levels was seen in diabetic animals.

3. Myo-inositol treatment partially prevented the depression of heart function of diabetic rats. The treatment was also effective in lowering plasma as well as myocardial triglycerols, cholesterol and phospholipid levels. The mechanism by which myo-inositol lowered lipids and improved heart function remains to be explored.

4. Myo-inositol failed to correct diabetes-induced hypothyroidism. However, improvement of cardiac function of diabetic rats was less pronounced with a combination treatment of both myo-inositol and T₃ compared to myo-inositol treatment alone, which may be related to the calorigenic and diabetogenic effects of thyroid hormones.

5. T₃ alone or in combination with myo-inositol had no effect on, or even further increased plasma and myocardial lipid levels of diabetic rats, even though heart function of these diabetic animals was partially improved.

In summary, myo-inositol appeared to control lipid metabolism as well as modify cardiac dysfunction of diabetic rats. However, myo-inositol-treatment in combination with T₃ did not lower the lipid levels of the diabetic rats, even though cardiac function of these diabetic animals
was partially improved. These findings do not support a causal relationship between elevated lipid levels and cardiac dysfunction in diabetic rats.
REFERENCES


