EFFECTS OF VANADIUM COMPOUNDS ON DIABETES INDUCED CHANGES IN STZ-DIABETIC RATS

By

JUN YAO

B.Sc., Beijing Medical University, P.R. China, 1990

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

Faculty of Pharmaceutical Sciences
Division of Pharmacology and Toxicology

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

April 1996

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
Vancouver, Canada

Date April 26. 96

ABSTRACT

PART I.

Acute and chronic response to vanadium following two methods of STZ-diabetes induction

Controversial reports on the efficacy and possible toxicity of vanadium obtained from various studies may be attributed to differences in the method of diabetes induction and/or to differences in animal strains. The objective of this study was to evaluate the contribution of these two factors to the effects of vanadium in the treatment of diabetes. Two methods of streptozotocin (STZ) induced diabetes in rats have been used for studying the antidiabetic effects of vanadium. One involves a single intravenous (i.v.) injection of 60 mg/kg STZ, and the other uses two subcutaneous (s.c.) injections of 40 mg/kg STZ, to either Wistar or Sprague Dawley (SD) rats. In a 7-week chronic study, SD rats appeared to develop a more severe diabetes (indicated by higher plasma cholesterol and higher fasting plasma glucose levels) following the single i.v. injection of STZ than rats made diabetic by two s.c. injections of STZ. Irrespective of the method of diabetes induction or the strain of rat, the responses of all the diabetic animals to vanadyl sulphate treatment were similar. In a 1-week acute study, Wistar diabetic rats were more responsive than SD diabetic rats to vanadyl sulphate and to lower doses (0.6 and 0.8 mmol/kg) of a new organic compound, bis(maltolato)oxovanadium(IV).

PART II.

Effect OF BMOV on diabetes-induced metabolic changes and cardiomyopathy in STZ-diabetic rats

Diabetic cardiomyopathy is a specific cardiac muscle disease characterized by a lower stroke volume, cardiac index and ejection fraction, and a higher left ventricular end diastolic pressure. Many factors contribute to the pathogenesis of diabetic cardiomyopathy including altered myocardial energy utilization, which is expressed as an excessive oxidation of free fatty acids, and/or an impaired glucose utilization by the heart. Insulin improves heart function in diabetes and its beneficial effects may be attributed to its action on cardiac metabolism. Insulin-mimetic vanadium compounds also reduce hyperglycemia and improve heart function in diabetes. The improved cardiac function has been proposed to be associated with an enhanced glucose oxidation in the diabetic heart. Therefore, the effect of bis(maltolato)oxovanadium (IV) (BMOV) on glucose oxidation in cardiac myocytes from control and acutely STZ-diabetic rats was examined in this study. Basal glucose oxidation was depressed in diabetic myocytes. Insulin stimulation of glucose oxidation was greater in control than diabetic myocytes. Incubation of cells with BMOV caused an increase in glucose oxidation only in cardiac myocytes from diabetic rats. Additionally, BMOV potentiated insulin-stimulated glucose oxidation in cardiac myocytes from diabetic rats. It is concluded that the beneficial effect of BMOV on cardiac function in the diabetic heart may be related to its effect in increasing glucose oxidation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Acetyl CoA	acetyl coenzyme A
ACC	acetyl-CoA carboxylase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATP	adenosine 5'-triphosphate
BMOV	bis(maltolato)oxovanadium (IV)
C	centigrade
CoA	coenzyme A
CPT	carnitine palmitoyl transferase
DCA	dichloroacetic acid
et al.	and others
Etomoxir	2-[6-(4-chlorophenoxy)hexyl]-oxirane-2-carboxylate
FABPs	fatty acid binding proteins
FFAs	free fatty acids
g	gram
Glut-4	glucose transporter-4
G-6-P	glucose-6-phosphate
i.v.	intravenous
i.p.	intraperitoneal
Joklik MEM	Joklik minimum essential medium
LCAC	long chain acylcarnitine
mg	milligram
min	minute
mL	milliliter
mM	millimolar

n	sample number
OGTT	oral glucose tolerance test
PDH	pyruvate dehydrogenase
PFK	phosphofructokinase
POCA	phenylalkyloxiranecarboxylic acid
s.c.	subcutaneous
s.e.m.	standard error of the mean
SD	Sprague Dawley
SR	sarcoplasmic reticulum
STZ	streptozotocin
TCA cycle	tricarboxylic acid cycle
TG	triglyceride
T_3	3,5,3'-triiodothyronine
U	units
Urea	plasma urea nitrogen
WKY	Wistar-Kyoto

ACKNOWLEDGMENTS

I wish to express my deepest gratitude to my supervisor, Dr. John H. McNeill, Dean, for his interest, knowledgeable guidance, encouragement and patience which enabled me to complete this work.

I wish to express my sincere thanks to Dr. Brian Rodrigues for his interest, valuable guidance and encouragement which helped me through all the difficulties and enabled me to finish this work successfully.

I would like to thank my supervisory committee, Dr. K.M. McErlane, Dr. R. Brownsey and Dr. R. Pederson for their valuable suggestion and constructive criticisms.

I thank Mary Battell and Dr. K.H. Thompson for their guidance, Stephanie Lee, Violet Yuen and Erika Vera for their technical support during the first part of this study. I thank Margaret Cam, Waiming Li, Nandakumar Sambandam, Subodh Verma and Sylvia Chan for their help in writing this thesis. The bis(maltolato)oxovanadium(IV) (BMOV) was synthesized by Lucio Gelmini in the laboratory of Dr. C. Orvig, department of Chemistry, UBC. This study was funded by the Medical Research Council of Canada.

DEDICATION

To my parents for all their love.

To Dr. Linfu Yao, for his encouragement and help in introducing me to Dr. John H. McNeill.

To my husband Wenji Yin, for his understanding, support, and excellent help while conducting the glucose oxidation assay.

PART I

Acute and Chronic Response to Vanadium Compounds Following Two Methods of STZ-Diabetes Induction

INTRODUCTION

1. Diabetes mellitus, insulin and vanadium

Diabetes mellitus is a group of syndromes characterized by altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from cardiovascular disease. Diabetes can be classified clinically into two major types: type-I or insulin dependent diabetes (IDDM) and type-II or non-insulin dependent diabetes mellitus (NIDDM). IDDM occurs as a result of a decrease in the circulating concentration of insulin (insulin deficiency). On the other hand, NIDDM is a result of a defect in the β-cell response to glucose in addition to a decrease in the response of peripheral tissues to insulin (insulin resistance).

Insulin was first discovered by Banting and Best in 1921 and has been the mainstay for treatment of Type-I diabetic patients and many Type-II diabetic patients who cannot be treated with oral hypoglycemics. Insulin lowers blood glucose level by inhibiting hepatic glucose production (glycogenolysis and gluconeogenesis) and stimulating glucose uptake and metabolism by muscle and adipose tissue. However, oral administration of insulin is ineffective in controlling diabetes, and the inconvenient daily injection and associated risk of hypoglycemia cause significant problems. Hence, the availability of orally active hypoglycemic agents would be of considerable importance in the treatment of diabetes. Vanadium, an insulin-mimetic agent, has been shown to have antidiabetic properties when given orally to streptozotocin (STZ)-diabetic (Heyliger *et al.* 1985), spontaneously diabetic (BB) (Battell *et a*l. 1992) and insulin-resistant Zucker fa/fa (Brichard *et al.* 1989) rats and recently to diabetic patients (Rossetti *et al.* 1994; Goldfine *et al.* 1994) and may prove to be a valuable adjunct to insulin in the treatment of diabetes mellitus.

2. Animal models of diabetes

STZ is an antibiotic complex which was isolated from *Streptomyces achromogenes* (Herr *et al.* 1960). STZ induced diabetes is primarily a result of highly specific cytotoxic action which causes rapid and irreversible necrosis of β-cells of the pancreatic islets (Junod *et al.* 1967). Histological studies of pancreas isolated from STZ-treated rats show a disruption of the islets and a marked decrease or complete absence of granules in the β-cells. STZ administration at doses exceeding 40 mg/kg results in a long-term, stable hyperglycemia (Ar'Rajab and Ahren 1993) and myocardial dysfunction can be seen after six weeks in rats made diabetic with 55 mg/kg STZ (Tahiliani *et al.* 1983). The caveat is that these animals are not dependent on exogenous insulin for survival and hence this STZ-diabetic model does not entirely resemble type-I insulin dependent diabetes.

3. Vanadium in experimental diabetes

Vanadium is a transition metal of the Vb group found in living organisms at concentrations which vary from pM to μM. It is a growth factor for various plants and is also considered essential for normal cell growth and development in some mammalian species. Vanadium is poorly absorbed from the gastrointestinal tract (Underwood 1977). Unabsorbed vanadium is excreted via the feces while homeostasis of absorbed vanadium is achieved by the kidney as excretion occurs in the urine. Absorbed vanadium can be stored in the bone, kidney and liver prior to excretion (Underwood 1977; Nielsen and Uthus 1990). In rats, the kinetics of vanadium turnover is best explained by a three component model. The plasma clearance curve of vanadium exhibits an initial half life of 15 minutes for the first 24

hours, a second half life of 14 hours over the next ten days and a third half life of 8.5 days for greater than 10 days (Sabbioni and Marafante 1978).

In 1985, Heyliger *et al.* first reported that treatment for 28 days with oral sodium orthovanadate produced euglycemia and prevented the decline in cardiac performance in STZ-diabetic rats. Subsequently, Meyerovitch *et al.* (1987) using metavanadate and Ramanadham *et al.* (1989) using vanadyl sulphate reported that these treatments were also effective in lowering plasma glucose levels in STZ diabetic rats. More recently, McNeill *et al.* (1992) showed that vanadium used in an organic form, bis(maltolato)oxovanadium(IV) (BMOV) also lowered plasma glucose levels and improved the depressed heart function in chronically STZ-diabetic animals.

It has been suggested that the glucose-lowering effect of vanadium is due to its insulin-mimetic properties, such as increasing glucose transport (Dubyak and Kleinzeller 1980), glycogen synthesis, glycolysis (Clark *et al.* 1985) and glucose oxidation (Shechter and Karlish 1980; Clark *et al.* 1985). Moreover, vanadium has also been reported to stimulate lipogenesis (Shechter and Ron 1986; Meyerovitch *et al.* 1987) and inhibit lipolysis (Duckworth *et al.* 1988; Ramanadham *et al.* 1989). These effects of vanadium may be due to an increased tyrosine phosphorylation of the insulin receptor (Tamura *et al.* 1984) and activation of subsequent events in the kinase phosphorylation cascade in a manner similar to the effects produced by insulin binding to the receptor. Vanadium has also been shown to be a phosphatase inhibitor (Swarup *et al.* 1982) and can increase phosphorylation of kinases distal to the insulin receptor (Shisheva and Shechter 1992; 1993).

Combining vanadium with other compounds has been used to form vanadium complexes with specific features. For example, Naglivan (Bis (cysteine, amide N-octyl) oxovanadium) (Cam *et al.* 1993) shows improved lipophilicity whereas peroxovanadate (Kadota *et al.* 1987; Shisheva and Shechter 1993; Posner *et al.* 1994) shows enhanced *in vitro* insulin-mimetic effects. BMOV is a new organic form of vanadium (Fig. 1). It is water soluble, neutrally charged, and readily available for gastrointestinal absorption when dissolved in drinking water (McNeill *et al.* 1992). BMOV is prepared nearly quantitatively (>90% yield) in water by combining maltol (3-hydroxy-2-methyl-4-pyrone) and vanadyl sulphate (2:1), raising the pH of the solution to 8.5, refluxing overnight, and collecting the deep purple-green compound which precipitates upon cooling. The increased absorption of vanadium after BMOV administration is reflected in higher tissue concentrations of vanadium than for a similar course of treatment with vanadyl sulphate (Yuen *et al.* 1993a). Additionally, in a 25-week study, diabetic rats given BMOV in the drinking water showed normalized glucose and lipid values and normal food and fluid intakes without an increase in insulin levels (Yuen *et al.* 1993b). Measurement of heart function indicated that BMOV treatment resulted in a significant correction of the heart dysfunction associated with STZ-induced diabetes (Yuen *et al.* 1993a). These results with BMOV are similar to previous reports using vanadyl sulphate to treat diabetic animals (Dai *et al.* 1994a).

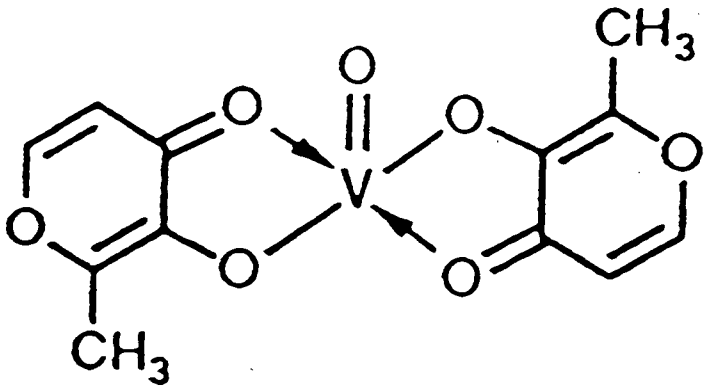


Fig. 1. Chemical structure of Bis(maltolato)oxovanadium.

4. Comparison of the anti-diabetic effects of BMOV and of vanadyl sulphate

Potency: In chronic studies, the dose of BMOV which was required to produce euglycemia was 0.2 mmol/kg/day (Yuen *et al.* 1993b), compared to 0.4 mmol/kg/day for vanadyl sulphate (Cam *et al.* 1993). In acute studies, vanadyl sulphate at the dose of 0.9 mmol/kg and BMOV at 0.55 mmol/kg given by oral gavage reduced plasma glucose levels to euglycemia in 50% of STZ-diabetic animals (Yuen *et al.* 1995). Hence, it appears that BMOV is more potent than vanadyl sulphate in producing euglycemia in STZ-diabetic rats.

Onset of action: When administered in drinking water, BMOV has a faster onset of action as normalization of plasma glucose levels occurs in 70% of STZ-diabetic animals within 24 hours (Yuen *et al.* 1993b). A similar degree of glucose lowering with inorganic vanadium was reported to occur within 4 to 7 days after the start of treatment (Meyerovitch *et al.* 1987; Sekar *et al.* 1990; Ramanadham *et al.* 1990). These results suggest that BMOV has a faster onset of action than vanadyl sulphate.

Withdrawal effect: STZ-diabetic rats which had been treated with vanadyl sulphate for a period of 3 weeks remained euglycemic for a period of 13 weeks following treatment withdrawal (Pederson *et al.* 1989). This sustained euglycemic effect was not observed following withdrawal of BMOV, after a treatment period of 10 weeks (Yuen *et al.* 1993b). In acute studies, the plasma glucose lowering effect of vanadyl sulphate occurred within 12 hours after which glucose levels reverted to pretreatment values by 24 hours. With BMOV, the euglycemic effect following acute administration remained for 3 to 14 weeks in some of the diabetic animals (Yuen *et al.* 1995). This prolonged euglycemic effect after a single dose of BMOV was

unexpected and contrary to results obtained after chronic treatment and subsequent withdrawal.

5. Possible toxicity of vanadium

Signs of vanadium toxicity have been reported in Sprague Dawley (SD) rats made diabetic with two s.c. injections of 40 mg/kg STZ. The toxic effects included decreased weight gain, renal impairment and death (Domingo *et al.* 1990; 1991a,b,c; 1992). This toxicity occurred regardless of the form of vanadium used (Domingo *et al.* 1991a,c). It should be noted that in these experiments, blood glucose levels were only partially controlled by the administration of three different vanadium compounds in the drinking water with vanadyl sulphate being the most effective after two weeks of treatment (Domingo *et al.* 1991a) and sodium metavanadate the most effective after a four week treatment period (Domingo *et al.* 1991b). When the compounds were administered by gavage, vanadyl (Domingo *et al.* 1992) and sodium metavanadate (Domingo *et al.* 1991b) were not effective in correcting blood glucose levels. Using the above data, it has been argued that potential toxicological complications could constitute a major problem for the use of vanadium in the treatment of diabetes.

In contrast, other laboratories have reported few signs of toxicity in studies with rats made diabetic with i.v. STZ injections. Meyerovitch *et al.* (1987) reported that treatment for 3 weeks with 0.2 mg/mL sodium metavanadate in 80 mM NaCl resulted in alleviation of the STZ-induced (55 mg/kg, i.v.) diabetes in male Wistar rats without toxic effects while diabetic rats receiving 0.8 mg/mL sodium metavanadate experienced hypoglycemia. Non-diabetic rats in this study did demonstrate elevated glutamic-oxaloacetic transaminase following vanadium

treatment. Gil *et al.* (1988) reported restoration of liver glucokinase activity and fructose 2, 6-bisphosphate levels in male SD rats made diabetic with 60 mg/kg STZ i.v. followed by treatment with 0.7 mg/mL sodium orthovanadate in 0.05 mg/mL NaCl solution for 14 days without apparent toxic effects. Strout *et al.* (1990) induced diabetes in male SD rats with 65 mg/kg STZ injected via the saphenous vein and then treated these rats with 20 mg/kg sodium orthovanadate injected intraperitoneally for 1 week without any toxicity. Valera *et al.* (1993) induced diabetes in male SD rats with a single i.v. injection of 60 mg/kg STZ. Treatment with 0.7 mg/mL of sodium orthovanadate in the drinking water for 15 days restored liver glucokinase mRNA concentrations. Serum alanine aminotransferase activity, which was elevated in the untreated diabetic rats, was also normalized by the vanadate treatment. Bollen *et al.* (1990) induced diabetes in male SD rats with 60 mg/kg STZ i.v. and then treated the animals with sodium orthovanadate for up to 18 days. The treatment resulted in normalization of the depressed glycogen synthase phosphatase and glucokinase activities in the isolated hepatocytes.

Reports from our laboratory have failed to detect significant toxic effects of vanadium treatment. In a year long toxicological experiment, male Wistar rats were made diabetic with 60 mg/kg STZ i.v. and treated with 0.5-1.25 mg/mL vanadyl sulphate in the drinking water. This resulted in an alleviation of the symptoms of diabetes (Dai *et al.* 1994 a, b, c). No effects of the one year vanadium treatment were found on the hematocrit, hemoglobin, erythrocyte count, reticulocyte percentage, platelet count, or leukocyte composition. In fact, the decreased leukocyte count in the untreated diabetic rats was restored to normal by treatment with vanadyl sulphate (Dai *et al.* 1994b). The treatment did not produce any changes in plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT) or urea. Rather, the elevated levels of ALT and urea found in the untreated

diabetic animals were prevented by vanadyl sulphate treatment (Dai *et al.* 1994c). No morphological abnormalities in brain, thymus, heart, lung, liver, spleen, pancreas, kidney, adrenal gland, or testis could be detected after vanadyl treatment (Dai *et al.* 1994c). Most importantly, treatment reduced the mortality rate from 60% in the untreated diabetic rats to 18% in the treated diabetic rats (Dai *et al.* 1994a).

The variable reports of toxic effects of vanadium could possibly be due to the different animal strains used and/or to differences in the protocols used for the induction of diabetes. This study investigated whether the different methods of inducing diabetes would result in any differences in the response of the diabetic SD animals to chronic vanadium treatment. Diabetes was induced by either one single i.v. injection of 60 mg/kg STZ or two s.c. injections of 40 mg/kg STZ. In addition, the contribution of strain differences to the response to vanadium was studied by inducing diabetes by the aforementioned methods in both SD and Wistar rats. These rats were then subjected to acute vanadium treatment using vanadyl sulphate and the more potent organic vanadium complex, BMOV.

Hypothesis:

Rats made diabetic by two s.c. injections of 40 mg/kg STZ would be more severely diabetic than rats made diabetic by a single i.v. injection of 60 mg/kg STZ and as a consequence, there would be a difference in their responses to vanadium treatment.

Rationale:

Reports of vanadium toxicity were obtained when using SD rats made diabetic by two s.c. injections of 40 mg/kg STZ, while no toxicity was observed in Wistar rats made diabetic by a single i.v. injection of 60 mg/kg STZ. Thus, the variable reports on the toxicity of vanadium could possibly be due to the severity of diabetes induced in the different animal strain used and/or to differences in the protocols used for the induction of diabetes.

Objectives:

To compare and investigate the antidiabetic response of oral vanadium treatment in Wistar and SD rats made diabetic by two different dosage schedules of STZ.

MATERIALS AND METHODS

1. Materials

1.1. Animals

Male Wistar and SD rats, weighing approximately 200 g were obtained from the Animal Care Center, University of British Columbia, Vancouver, B.C., Canada. They were maintained on a 12 hour light/dark cycle and were allowed free access to standard laboratory food (Purina rat chow) and water.

1.2. Chemicals

Sigma Company, St. Louis, MO, USA.

Streptozotocin, kits for plasma urea nitrogen, creatinine, aspartate aminotransferase and alanine aminotransferase.

Ayerst Lab, Montreal, Que., Canada

Fluothane.

Boehringer Mannheim GmbH Diagnostica, Germany

Glucose, triglyceride, cholesterol and free fatty acid kits.

Fisher Scientific Co., Fair Lawn, NJ, U.S.A.

Vanadyl sulphate ($VOSO_4 \cdot nH_2O$)

Linco Research, Inc., St. Charles, MO, U.S.A.

Rat plasma insulin assay kits.

Ames, Miles Lab, Elkhart, IN, U.S.A.

Glucostix reagent strips and Glucometer II.

2. Methods

2.1. Induction of diabetes

Diabetes was induced by two methods. One group of rats was made diabetic by two s.c. injections of 40 mg/kg STZ in 0.1 M cold citrate buffer (pH 4.5) at day 0 and 3 (12:00 noon); the other group was made diabetic by a single i.v. injection of 60 mg/kg STZ in the same buffer at day 3 into the tail vein. Both the i.v. and s.c. injections were made under light halothane anesthesia. Diabetes was confirmed at 72 hours after the final STZ injection by the presence of hyperglycemia (>13.8 mM) in blood samples obtained from tail vein, using Glucostix reagent strips read by a Glucometer II.

2.2. Chronic vanadyl sulphate treatment

In order to eliminate the possible contributions from animal strain difference, only SD rats were used in the chronic study. The rats receiving a single i.v. injection of 60 mg/kg STZ were randomly divided into D1 untreated (D1, n=6) and D1 treated (D1T, n=6) groups. The rats receiving two s.c. injections of 40 mg/kg STZ were randomly divided into D2 untreated (D2, n=5) and D2 treated (D2T, n=5) groups. Control rats were divided into control untreated (C, n=6) and control treated

groups (CT, n=6). All treated rats were given vanadyl sulphate in drinking water beginning at one week after the final STZ injection at a concentration of 0.5 mg/mL for the first 3 days, followed by 0.75 mg/mL for the remainder of the 7-week treatment period. This dose of vanadyl sulphate was chosen based on a previous report which demonstrated pronounced glucose lowering effect in STZ-diabetic rats. (Cam *et al.* 1993).

Body weight, food and fluid intake were monitored daily. Blood samples were taken from the tail vein during the treatment period to monitor plasma glucose by the glucose oxidase method, plasma insulin using rat insulin standard and an antibody prepared against rat insulin, and plasma vanadium by graphite furnace atomic absorption spectrophotometry (Perkin Elmer 2380, with deuterium lamp background correction) as described previously (Mongold *et al.* 1990). At the time of termination, blood samples were collected by cardiac puncture.

An oral glucose tolerance test (OGTT) was conducted after 5 weeks of treatment. All animals were fasted overnight and an oral glucose dose (1g/kg as a 40% solution) was administered to conscious animals by oral gavage. Blood samples were obtained from the tail vein before and at 10, 25, 35 and 60 minutes after glucose administration in order to monitor plasma glucose and insulin levels.

2.3. Acute vanadium treatment

Diabetes was induced by the above two methods (D1 and D2) as previously described in both SD and Wistar rats. One week after the final STZ injection, the conscious diabetic animals were given a single dose of vanadyl sulphate (0.9 mmol/kg) or BMOV (0.6 mmol/kg in 3% gum arabic suspension) by oral gavage.

Doses were chosen based on previous studies in which oral gavage administration of a single dose of vanadyl sulphate (0.9 mmol/kg) or BMOV (0.55 mmol/kg) produced euglycemia in 50% of diabetic-Wistar animals (Yuen *et al.* 1995). Plasma glucose levels were monitored before and at 4, 6, 8, 12, 24 and 48 hours after treatment. The animals which became euglycemic (≤ 9 mM) were considered to have responded to treatment. Rats that did not achieve euglycemia were gavaged a second time at the same or higher dose at 48 hours after the initial dose. The non-responders to the second dose were given a third higher dose at 96 hours.

2.4. Statistical analysis

Statistical differences among the different groups were determined by one-way analysis of variance (ANOVA) followed by Fisher's LSD multiple-comparison test. The χ^2 test was also used for making comparisons between groups of rats used for the acute study. The level of significance was set at $p<0.05$.

RESULTS

1. Chronic vanadium treatment

Body weight, food and fluid intake, vanadium dose and plasma vanadium (Table 1)

After 7 weeks of treatment, the body weight of CT group was reduced compared to the C group. All diabetic groups had lower body weights than did the C group. Both diabetic untreated groups (D1, D2) exhibited hyperphagia and polydipsia. Vanadyl treatment normalized food and fluid intake in both diabetic treated groups (D1T, D2T). The dose (mmol/kg) of vanadium in D1T and D2T groups was not different but was higher than in the CT. Plasma vanadium levels of the CT, D1T and D2T groups were not different from each other.

Plasma glucose and insulin

Prior to treatment, all diabetic groups showed evident hyperglycemia (Fig. 1). Following treatment, glucose levels of D1T and D2T were significantly reduced, but did not reach control values. Vanadyl did not affect the glucose level of CT. No significant differences were seen between D1 and D2, nor between the D1T and D2T groups.

Prior to treatment, the plasma insulin values (ng/mL) were lower in D1 (2.31± 0.29, n=12) and D2 (2.36±0.24, n=10) groups than in the C group (3.67±0.43, n=12). After 3 weeks of treatment, the plasma insulin level in the CT group was reduced as compared to the C group (C=3.33±0.53, n=6; CT=2.08±0.45, n=6). The

insulin levels were not different between D1 and D2 (D1=0.55±0.10, n=6; D2=0.74± 0.10, n=5), nor between D1T and D2T (D1T=1.87±0.63, n=5; D2T=1.52±0.33, n=5).

Oral glucose tolerance test

D1T and D2T had lower plasma glucose levels as compared to D1 and D2 at all time points. However, peak glucose levels were higher in D1T and D2T when compared to C and CT (Fig. 2A). The glucose level of the CT group was not different when compared to the C group. No significant differences were seen between D1 and D2 animals except that fasted glucose values were lower in D2 than in D1 rats. No significant differences were seen between D1T and D2T animals. The plasma insulin level of the C group was significantly higher than all other groups prior to glucose challenge (Fig. 2B). CT demonstrated an insulin release pattern similar to that of the untreated control, but the insulin levels were significantly lower than C for all time points except at 60 minutes. None of the diabetic and diabetic-treated animals demonstrated an insulin response to the glucose challenge and the insulin levels of all diabetic groups were not different from each other.

Plasma lipids

At termination, there was no significant difference in plasma FFAs between any of the groups (Table 2). Plasma triglyceride levels (mM) were significantly higher in D1 (D1=2.92±0.64, n=6) but not in D2 (D2=2.08±0.33, n=5) when compared to the C group (C=1.18±0.15, n=6). Vanadyl restored the plasma triglyceride of D1T group to normal (D1T=1.52±0.42, n=5). Triglyceride levels were not significantly different between D1 and D2, nor between the D1T and D2T

groups. The plasma cholesterol levels (mM) were higher in D1 than in C (D1=1.89± 0.19, n=6; C=1.07±0.07, n=6) whereas cholesterol levels in D2 were not elevated (D2=1.19±0.055, n=5) over control. Vanadyl restored the plasma cholesterol levels of D1T to normal (D1T=1.08±0.11, n=5). Plasma cholesterol levels were higher in the D1 group as compared to D2 group (D1=1.89±0.19, n=6; D2=1.19±0.05, n=5).

Liver and kidney function

There was no significant difference between any of the groups when plasma urea nitrogen, creatinine and AST levels (Table 2) were measured. The D1, but not the D2 group had significantly higher plasma ALT levels (SF units/mL) than the control group (C=37.8±2.2, n=6; D1=53.2±7.5, n=6; D2=42.8±4.0, n=5). Vanadyl reduced the ALT level to control level in D1T group (D1T=26.9±1.8, n=5).

2. Acute vanadium treatment

Vanadyl sulphate and BMOV were administered by oral gavage to both SD and Wistar rats made diabetic by the two methods used in the chronic study outlined above. Both compounds resulted in acute glucose lowering effects in the two strains of rats. In these experiments, rats which did not show euglycemia following the initial administration of the compound, received a second dose at the same or higher concentration, 48 hours after the initial dose. The number and percentage of euglycemic (≤ 9 mM) rats after acute gavage with vanadyl sulphate and BMOV in Wistar and SD rats are shown in Table 3A. For vanadyl sulphate in the Wistar rats, 7 out of 9 D1 and all the D2 (n=8) animals achieved euglycemia after 3 doses of 0.9, 0.9 and 1.4 mmol/kg; in the SD rats, 4 out of 20 D1 and 3 out of 10 D2 animals achieved euglycemia after 3 doses of 0.9, 1.4 and 2.0 mmol/kg. The

three doses of BMOV administered were 0.6, 0.8 and 1.0 mmol/kg. In Wistar rats, 8 out of 9 of D1 and 9 out of 10 D2 rats achieved euglycemia. In SD rats, 15 out of 19 D1 and all the D2 (n=10) rats achieved euglycemia.

The acute glucose-lowering effects of vanadyl sulphate or BMOV were not different between D1 and D2 animals in both animal strains. Therefore, the data were combined to compare the difference responses between the two animal strains (Table 3B). With vanadyl sulphate, 15 out of 17 Wistar and only 7 out of 30 SD rats achieved euglycemia. There was a significant difference between the two animal strains in their responses to vanadyl sulphate. Therefore, Wistar rats were more responsive to the acute administration of vanadyl sulphate than SD rats. After the administration of BMOV at lower doses of 0.6 and 0.8 mmol/kg, 17 out of 23 Wistar (74%) and 20 out of 45 Sprague Dawley (44%) rats responded. Thus, it appeared that Wistar rats were more responsive to BMOV than SD rats at lower doses. This difference in responsiveness was lost at higher dose of BMOV (1.0 mmol/kg) as 17 out of 19 Wistar (89%) and 25 out of 29 SD (86%) rats achieved euglycemia.

Figure 1

Effects of STZ-diabetes and vanadium treatment on plasma glucose levels. Diabetes was induced with either a single tail vein injection of 60 mg/kg STZ (D1) or with two subcutaneous injections of 40 mg/kg STZ (D2). Rats were monitored during a 7-week treatment period with vanadyl sulphate beginning at 0.5 mg/mL for 3 days followed by 0.75 mg/mL for the remainder of the treatment period.

Results are expressed as mean ± S.E.M.

* denotes significantly different from control groups.

# denotes significantly different from diabetic untreated groups.

FIGURE 1

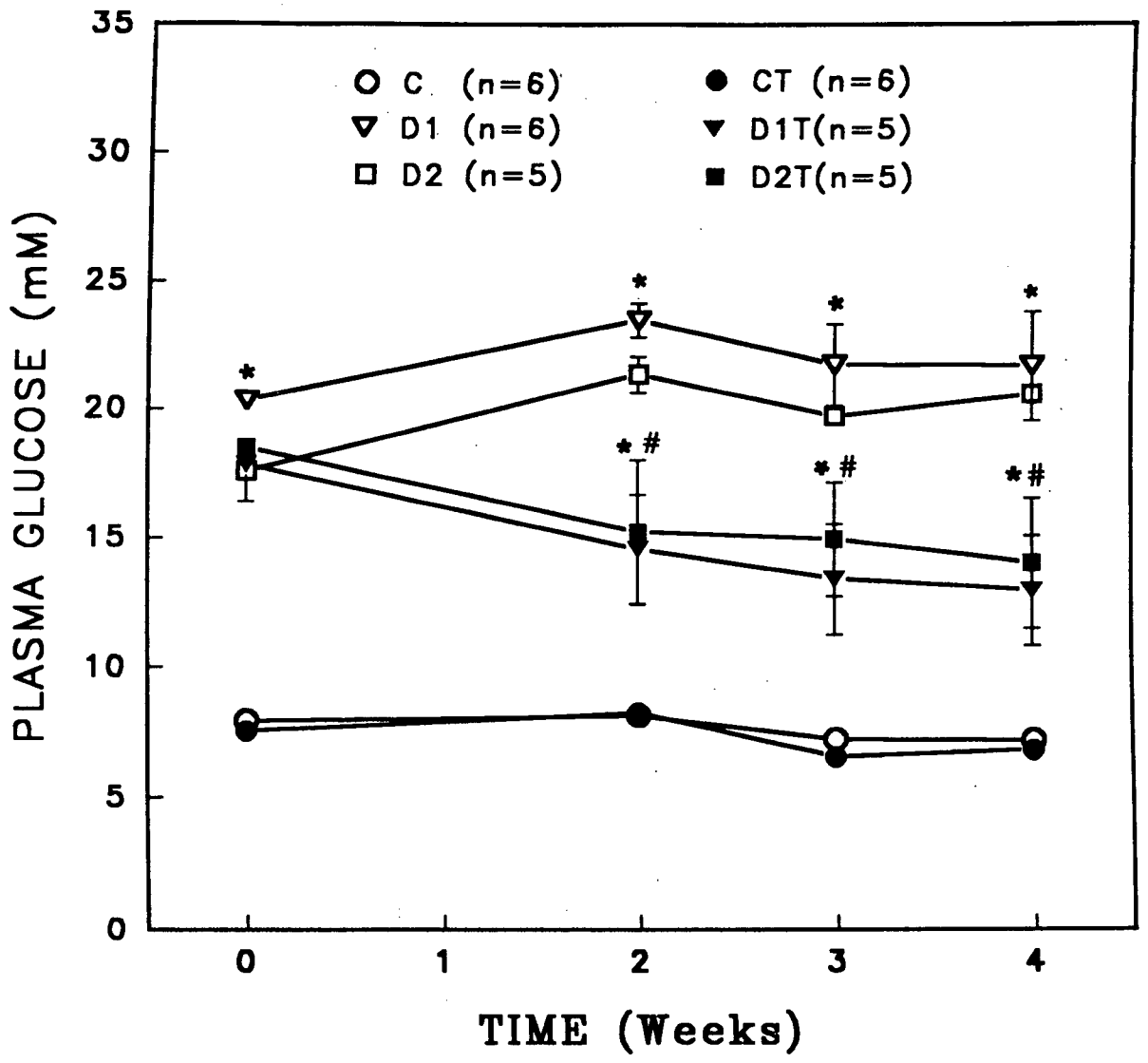

C (n=6)
D1 (n=6)
D2 (n=5)
CT (n=6)
D1T(n=5)
D2T(n=5)
PLASMA GLUCOSE (mM)
TIME (Weeks)
0
5
10
15
20
25
30
35
0
1
2
3
4
*
*#

Figure 2

Effect of STZ-diabetes and vanadium treatment on an oral glucose tolerance test conducted after 5 weeks of vanadyl sulphate treatment described in Fig. 1. Overnight fasted animals received 1 g/kg glucose by oral gavage. Plasma glucose and insulin levels were monitored before and at 10, 25, 35, and 60 minutes after glucose administration.

Results are expressed as mean ± S.E.M.

* denotes significantly different from D2 group; $p<0.05$.

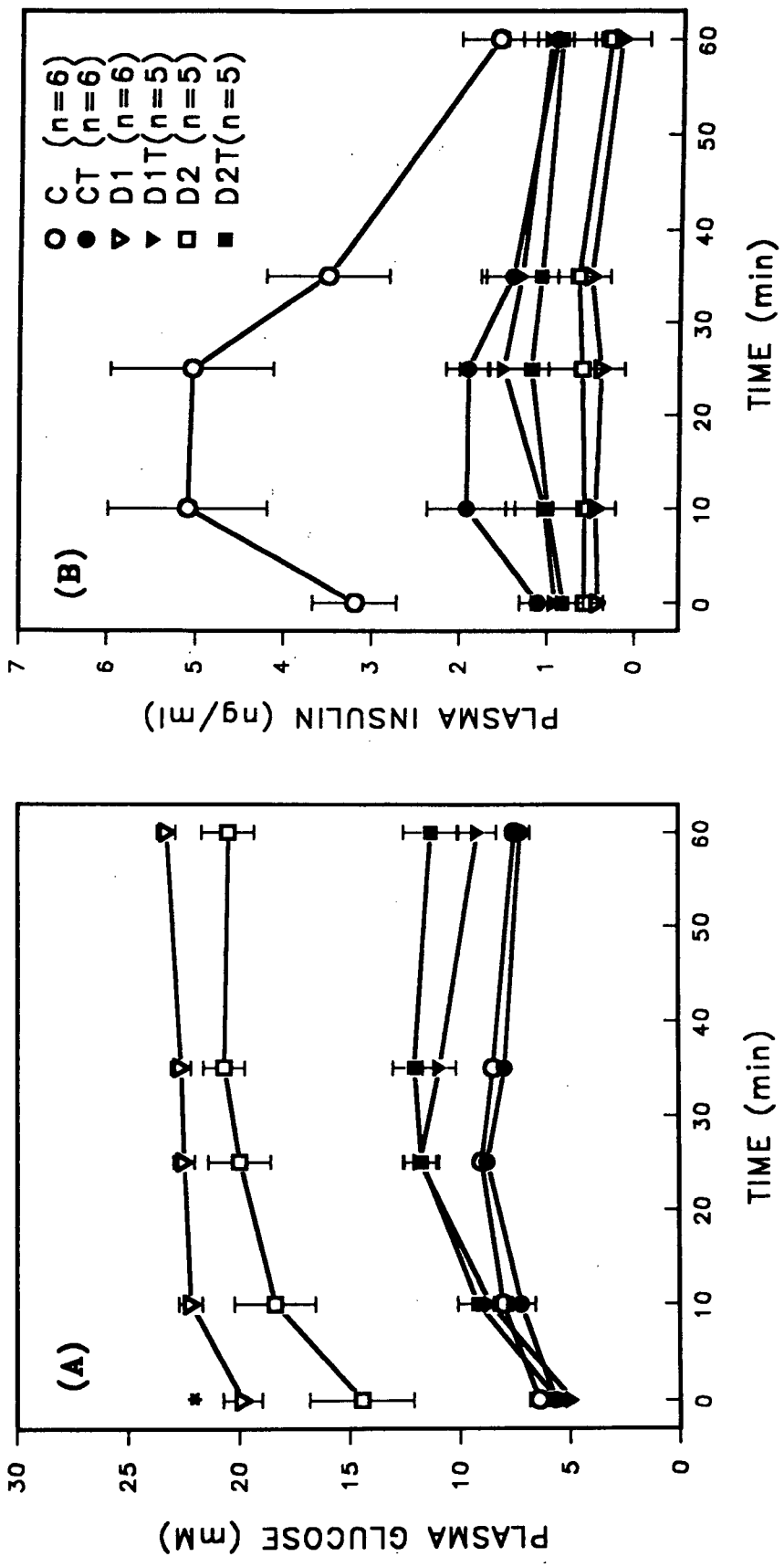

(A)
PLASMA GLUCOSE (mM)
TIME (min)
(B)
PLASMA INSULIN (ng/ml)
TIME (min)
C (n=6)
CT (n=6)
D1 (n=6)
D1T (n=5)
D2 (n=5)
D2T (n=5)

FIGURE 2

TABLE 1

Body weight, food and fluid intake, vanadium dose and plasma vanadium after 7 weeks of treatment. (Sprague Dawley rats)

	C (n=6)	CT (n=6)	D1 (n=6)	D1T (n=5)	D2 (n=5)	D2T (n=5)
Body Weight (g)	527±12	455±11*	341±8*	409±33*#	344±16*	376±38+*
Food Intake (g)	38±2	38±1	67±2*	31±1+#	64±1*	29±1+#
Fluid Intake (mL)	58±4	38±1*	343±9*	59±5#	342±11*	58±6#
Vanadium Dose (mmol/kg)	--	0.25±0.01	--	0.47±0.12+	--	0.56±0.09+
Plasma Vanadium (μg/mL)	--	0.24±0.02	--	0.27±0.08	--	0.22±0.08

Plasma Vanadium was not measured in untreated rats.

Data is expressed as mean ± S.E.M.

* Significantly different from the C group.

\# Significantly different from the untreated-diabetic control.

\+ Significantly different from the CT group ($p<0.05$).

TABLE 2

Plasma parameters at time of termination (Sprague Dawley Rats)

	C (n=6)	CT (n=6)	D1 (n=6)	D1T (n=5)	D2 (n=5)	D2T (n=5)
Free fatty acids (mM)	0.65±0.18	0.39±0.08	0.75±0.27	0.50±0.09	0.33±0.03	0.54±0.08
Triglyceride (mM)	1.18±0.15	1.04±0.10	2.92±0.64*	1.52±0.42#	2.08±0.33	1.41±0.19
Cholesterol (mM)	1.07±0.07	1.12±0.07	1.89±0.19*	1.08±0.11#	1.19±0.05#	1.08±0.10
Urea (mg/dL)	19.08±0.56	18.02±0.77	17.27±0.17	16.89±2.01	20.10±1.06	18.69±0.56
Creatinine (mg/dL)	0.19±0.04	0.20±0.03	0.15±0.03	0.10±0.04	0.11±0.01	0.10±0.05
AST (SF Units/mL)	84.8±6.5	75.5±9.3	95.5±5.7	74.9±4.8	82.5±4.5	77.3±9.1
ALT (SF Units/mL)	37.8±2.2	23.6±1.5	53.2±7.5*	26.9±1.8#	42.8±4.0	29.0±5.8

Results are expressed as mean ± S.E.M.

* Significantly different from the C group.

\# Significantly different from D1 ($p<0.05$).

TABLE 3A

Number and percentage of euglycemic rats following acute gavage treatment with vanadyl sulphate or BMOV. (Euglycemia is defined as plasma glucose ≤ 9 mM).

	Doses (mmol/kg)	Wistar D1	Wistar D2	Doses (mmol/kg)	Sprague Dawley D1	Sprague Dawley D2
VS	0.9	6/9	3/8	0.9	2/20	1/10
	0.9	0/3	2/5	1.4	0/18	1/9
	1.4	1/3	3/3	2.0	2/18	1/8
	Total	7/9 (78%)	8/8 (100%)	Total	4/20 (20%)	3/10 (30%)
BMOV	0.6	7/9	8/10	0.6	9/19	4/10
	0.8	1/2	1/2	0.8	4/10	3/6
	1.0	0/1	0/1	1.0	2/6	3/3
	Total	8/9 (89%)	9/10 (90%)	Total	15/19 (79%)	10/10 (100%)

TABLE 3B

Comparison of the number and percentage of euglycemic rats following acute gavage treatment with vanadyl sulphate or BMOV (Euglycemia is defined as plasma glucose ≤ 9 mM).

	Doses (mmol/kg)	Wistar	Doses (mmol/kg)	Sprague Dawley
VS	0.9	9/17	0.9	3/30
	0.9	2/8	1.4	1/27
	1.4	4/6	2.0	3/26
	Total	15/17 (88%)	Total	7/30 (23%)*
BMOV	0.6	15/19	0.6	13/29
	0.8	2/4	0.8	7/16
	1.0	0/2	1.0	5/9
	Total	17/19 (89%)	Total	25/29 (86%)

* Significantly different from Wistar rats ($p < 0.05$).

DISCUSSION

The variable reports on the possible toxicity of vanadium in the treatment of diabetes may be due to the different animal strains used, or to different STZ-diabetes induction methods. This study was designed to compare two methods of STZ-diabetes induction by comparing the severity of diabetes achieved and the responses to vanadium treatment.

1. Toxic effects of vanadium

During the chronic study, hypoglycemia (<3 mM) was observed in 1 out of 6 D1T animals. This animal showed symptoms of lethargy, weight loss and refusal to eat and drink. Treatment of this hypoglycemia included oral gavage administration of 5% glucose solution, withdrawal from treatment, and the use of 5% glucose solution as drinking water. The animal recovered quickly and was restarted on a lower vanadyl concentration (0.25 mg/mL). However, this animal was excluded from the study as hypoglycemia recurred and treatment had to be terminated. Near the end of this study, 1 out of 6 D2T rats also showed symptoms of hypoglycemia. Treatment was withdrawn immediately and the rat recovered. The hypoglycemia seen in 2 of 12 diabetic treated rats in this study may have been the cause of the mortality seen among vanadium treated rats reported by other workers (Domingo *et al.* 1991a,b,c; 1992). However, most death associated with vanadium ingestion in diabetic animals are more likely due to a decreased fluid intake (Ramanadaham *et al.* 1989). All other treated animals were healthy during the 7-week treatment period without overt indications of toxicity. Moreover, vanadium treatment did not elevate the urea, creatinine, ALT or AST levels in all the treated groups, but normalized the elevated ALT level in D1T group to control level. The lack of toxic effects of

vanadium seen in our study agrees with the results previously reported in a year long study by Dai *et al.* 1994 (a,b,c) and contrasts sharply with the toxic effects reported by Domingo *et al.* (1991a,b,c; 1992) in rats of the same strain made diabetic by the same method.

We had originally hypothesized that the toxicity reported following vanadium treatment of diabetic rats might be due to a more severe form of diabetes induced by two injections compared to that induced by a single injection. However, we found that rats made diabetic by two s.c. injections of 40 mg/kg STZ were not more severely diabetic than those made diabetic by a single i.v. injection of 60 mg/kg STZ. In fact, several parameters such as fasted plasma glucose and plasma cholesterol levels at termination indicated that rats made diabetic by a single i.v. injection of 60 mg/kg STZ were more severely diabetic than those made diabetic by two s.c. injections of 40 mg/kg STZ.

2. Differences between rat strains

Different strains of rats might respond differently to induction of diabetes with STZ, and hence might respond differently to treatment with insulin-mimicking agents. In a previous experiment, the severity of diabetes was examined in both SD and Wistar rats made diabetic with a single i.v. injection of 60 mg/kg STZ (Dai *et al.* unpublished data). No differences were seen between strains in body weight, food or fluid intake, plasma lipid levels or plasma insulin measurement at various time points during a 7-week study. Plasma glucose was not different in weekly blood samples except following an overnight fast when the Wistar diabetic rats had a plasma glucose of 7.69±1.28 mM, n=10, while the SD diabetic rats had a value of 16.61±1.28, n=9. However, in other studies of STZ-induced diabetes in Wistar rats,

overnight fasting has resulted in only moderate reduction in glucose levels (Pederson *et al.* 1989; Yuen *et al.* 1993b). These results indicate that diabetes induction with STZ does not result in a consistent diabetic state in all animals. Factors such as the particular batch of STZ, the length of time between injection and last meal of the rats, the skill of operators, and the instability of STZ result in a model which, while relatively homogeneous, is not completely consistent from experiment to experiment.

In the acute study, in diabetic SD rats, vanadyl sulphate at doses of 0.9, 1.4 and 2.0 mmol/kg reduced plasma glucose levels to euglycemia in 20% of D1 (n=20) and 30% of D2 (n=10) rats (Table 3A). Since the last dose had exceeded the reported LD_{50} value of vanadium in rats (1.77 mmol/kg) (Llobet and Domingo 1984), it was not likely that higher doses would have produced glucose lowering effects without toxic effects. On the other hand, in diabetic Wistar rats, vanadyl sulphate reduced glucose levels in 78% of D1 (n=9) and 100% of D2 (n=8) animals to euglycemia at lower doses (0.9, 0.9 and 1.4 mmol/kg). This may indicate that Wistar rats responded better than SD rats to acute vanadyl treatment. A possible explanation for the above results is that vanadium may be better absorbed by Wistar rats compared to SD rats. With higher dose of BMOV (1.0 mmol/kg), both Wistar and SD rats responded similarly to treatment. However, at lower doses (0.6 and 0.8 mmol/kg), 74% of Wistar and 44% of SD rats responded to BMOV indicating that Wistar rats respond better than SD rats to vanadium compounds.

Our data do demonstrate chronic effectiveness of vanadium in SD rats as was demonstrated by Domingo *et al.* 1991 (a,c). However, reports by the same group have suggested that vanadyl sulphate is ineffective in lowering plasma glucose when administered by chronic gavage treatment to SD rats (Domingo *et al.*

1992). The doses administered were 25 to 75 mg/kg/day, while the doses used in our acute experiment were 195 mg/kg to 434 mg/kg. Moreover, in chronic experiments using Wistar rats, vanadyl sulphate was effective at about 100 mg/kg/day (Cam *et al.* 1993). Thus, the lack of effects following chronic gavage treatment reported by Domingo *et al.* (1992) may be attributed to the following: lower absorption of vanadyl sulphate in SD rats as compared with Wistar rats; doses that were too low to be effective (1/4 to 1/2 of the doses by Yuen *et al.* 1995), differences in the severity of diabetes and the amount of residual circulating insulin (Cam *et al.* 1995) between diabetic rats in the two studies.

Regardless of the method of diabetes induction, antidiabetic effects of chronic vanadium treatment were seen in both groups of diabetic animals with chronic treatment. Following acute treatment, the effectiveness of vanadyl sulphate was greater in Wistar diabetic-rats than in SD diabetic-rats while BMOV was equally effective in both strains.

CONCLUSIONS

1. Chronic oral administration of vanadyl sulphate at the concentration of 0.75 mg/mL was effective in the treatment of SD rats made diabetic either by one single i.v. injection of 60 mg/kg STZ or by two s.c. injections of 40 mg/kg STZ without apparent toxicity.

2. SD rats appeared to develop a more severe diabetes (indicated by plasma cholesterol and fasting plasma glucose levels) when made diabetic by a single i.v. injection of 60 mg/kg STZ compared to rats made diabetic by two s.c. injections of 40 mg/kg STZ. However, their responses to chronic vanadyl sulphate treatment were not different.

3. Wistar diabetic rats were more responsive than SD diabetic rats to acute treatment with vanadyl sulphate, or lower doses of BMOV (0.6 and 0.8 mmol/kg).

PART II

Effect of BMOV on Diabetes-Induced Metabolic Changes and Cardiomyopathy in STZ-Diabetic Rats

INTRODUCTION

1. Diabetes induced cardiomyopathy

The occurrence of coronary and congestive heart disease is much greater in diabetes (Kannel *et al.* 1974). Factors that appear to largely account for this increased incidence of cardiovascular dysfunction during diabetes include atherosclerosis of the coronary arteries, macroangiopathy and autonomic neuropathy (Ledet *et al.* 1979). The existence of a specific cardiac muscle disease was first suggested by Rubler *et al.* (1972) who found that some diabetic patients suffered from congestive heart failure in the absence of atherosclerotic, valvular, congenital and hypertensive heart disease. This was confirmed by Hamby *et al.* (1974) based on the fact that diabetic patients exhibited clinical hemodynamic evidence of cardiac dysfunction in the presence of normal coronary arteries. The finding that diabetic heart disease can occur even in the presence of normal coronary arteries indicates that factors other than abnormal blood vessels could be involved in the pathogenesis of heart disease. The term, "diabetic cardiomyopathy" has been used to define this specific cardiac contractile dysfunction in the absence of coronary artery disease (Fein and Sonnenblick 1985; Galderisi *et al.* 1991). Diabetic cardiomyopathy is associated with low stroke volume, cardiac index and ejection fraction, and high left ventricular end diastolic pressure (Hamby *et al.* 1974; Regan *et al.* 1977; D'Elia *et al.* 1979).

2. Animal models of diabetic cardiomyopathy

Streptozotocin (STZ) diabetes model has been used as a diabetic cardiomyopathy model. Similar to clinical diabetic cardiomyopathy, both systolic

and diastolic functions are impaired in STZ-diabetic animals. Myocardial dysfunction is normally seen in six-week STZ-diabetic rats (Tahiliani *et al.* 1983) and includes diminished contractile output and stroke work at high filling pressure, decrease in peak left ventricular systolic pressure, and a delay in diastolic relaxation (Penpargkul *et al.* 1980). Myocardial muscle mechanics are also characterized by a significant delay in relaxation and a reduced velocity of contraction (Fein *et al.* 1980) when using papillary muscle from left ventricle of chronic diabetic rats. An interesting feature of this diabetic cardiomyopathy is that metabolic abnormalities may be predominant in the early acute phases of diabetes and cardiac function can be normalized by perfusion of acutely diabetic rat hearts with high concentrations of glucose and insulin (Meerson 1969; Ingebretsen *et al.* 1980). However, heart performance could not be restored in a similar manner in chronically diabetic hearts (Rosen *et al.* 1986).

3. Pathogenesis of diabetic cardiomyopathy

Diabetic cardiomyopathy may occur as a direct consequence of the insulin deficient state on myocardial cell function. The most successful treatment in preventing and reversing cardiac abnormalities associated with diabetes is insulin. However, insulin treatment is not always totally effective, especially in reversing the long term effects of diabetes on the heart (Tahiliani *et al.* 1983). Clinical studies have also indicated that myocardial abnormalities remain despite insulin treatment and rigid glucose control (The University Group diabetes Program 1975). Diabetic cardiomyopathy can occur due to a combination of several long-term irreversible changes. These include changes in:

3.1. Chronic changes

Compliance:

Hearts from diabetic dogs (Regan *et al.* 1974) and from diabetic patients at autopsy (Regan *et al.* 1977) were shown to accumulate periodic acid Schiff (PAS)-positive materials and collagen which could lead to increased stiffness of ventricular wall and decreased compliance of the myocardium.

Contractile protein distribution and myosin ATPase activity:

The changes in the velocity of systolic contraction are associated with alterations in myosin ATPase activity and myosin isoenzyme predominance (Schwartz *et al.* 1981). The myosin ATPase activity in diabetic hearts was decreased by 40-50% (Malhotra *et al.* 1981; Dillmann 1980). The depressed myosin ATPase activity was caused by myosin isoenzymes shifting from a more active V1 form to a less active V3 form as a consequence of diabetes induced hypothyroidism (Malhotra *et al.* 1981; Dillmann 1980). The depressed myosin ATPase activity can be prevented by replacement with pharmacological doses of thyroid hormone (Dillmann 1982). However, the reversal was incomplete and physiological levels of triiodothyronine administration to diabetic rats did not reverse the depressed cardiac function to normal (Tahiliani and McNeill 1985).

Sarcoplasmic reticulum function:

Diastolic relaxation of the heart is mediated to a large extent by the uptake of Ca^{2+} into the sarcoplasmic reticulum (SR). The decreased speed of diastolic

relaxation in the diabetic heart can be linked to a decrease of Ca^{2+}-ATPase activity of the SR, and a resultant decrease of Ca^{2+} transport into the SR (Penpargkul *et al.* 1981; Ganguly *et al.* 1983). As a consequence, the Ca^{2+} available for release is decreased. The decreased SR Ca^{2+}-ATPase activity is related to an elevation of long chain acyl carnitines in the SR membrane (Lopaschuk *et al.* 1982) and alteration in lipid composition of SR (Ganguly *et al.* 1983).

Sarcolemmal membrane function:

Ca^{2+} transport out of diabetic cardiac cells is impaired in chronic STZ-diabetic rats. Diabetes is associated with alterations in sarcolemmal membrane function, and includes a decrease in the ability of sarcolemmal membranes to bind Ca^{2+} (Pierce *et al.* 1983) and depressed Na^{+}-K^{+}-ATPase (Pierce and Dhalla 1983) and Ca^{2+} pump activity (Heyliger *et al.* 1987). All of these factors contribute to the impaired contraction and relaxation in diabetic heart.

Circulating plasma lipids:

Elevated circulating plasma lipids may alter the integrity of membranes and enzyme activities, and result in cardiac dysfunction in STZ-diabetic rats. For example, Wistar and Wistar-Kyoto (WKY) rats injected with identical doses of STZ demonstrate elevated blood glucose levels. However, only Wistar diabetic rats exhibited elevated levels of circulating lipids. Moreover, the depressed cardiac function seen in Wistar diabetic rats was not observed in WKY diabetic rats (Rodrigues *et al.* 1986). Treatment of STZ-diabetic Wistar rats with hydralazine reduced the elevated plasma triglyceride levels with a subsequent improvement in cardiac function (Rodrigues *et al.* 1986; Burns *et al.* 1991). These results suggest

that hyperlipidemia is an important factor in altering cardiac function in STZ-diabetic rats. However, other drugs like clofibrate, verapamil, prazocin and enalapril reduced plasma triglyceride levels but did not prevent the onset of heart dysfunction in chronically diabetic rats (Rodrigues *et al.* 1994).

3.2. Acute changes

In the early acute phases of diabetes, metabolic abnormalities may be predominant. It has been proposed that metabolic derangements in fuel supply and utilization by heart tissue may play a crucial role in the genesis of diabetic cardiomyopathy (Rodrigues and McNeill 1992). For example, maximal contractile performance of cardiac tissue depends on an adequate supply of high-energy phosphate compounds. The ATP level is decreased by 30% in diabetic rat hearts (Jenkins *et al.* 1988). The creatinine kinase enzyme system plays an important role in high-energy phosphate transduction in cardiac tissues. The total cardiac creatinine kinase activity is decreased by 30% in diabetic heart (Dillmann *et al.* 1988). Hence, decreased ATP levels and an impaired creatinine kinase system may contribute to the diminished contractile performance of the diabetic heart.

Other metabolic derangements are expressed as an excessive oxidation of FFAs and/or impaired glucose oxidation. Mitochondrial generation of ATP in the heart is through oxidation of various substrates including free fatty acids (FFAs), glucose, lactate and ketone bodies (Fig. 1). FFAs are the principal form utilized by the heart muscles for energy production (van der Vusse *et al.* 1992). FFAs are supplied to the heart either through lipolysis of endogenous triglyceride stores by triglyceride lipase or through lipolysis of triglyceride from the blood by lipoprotein lipase, or as FFA bound to albumin.

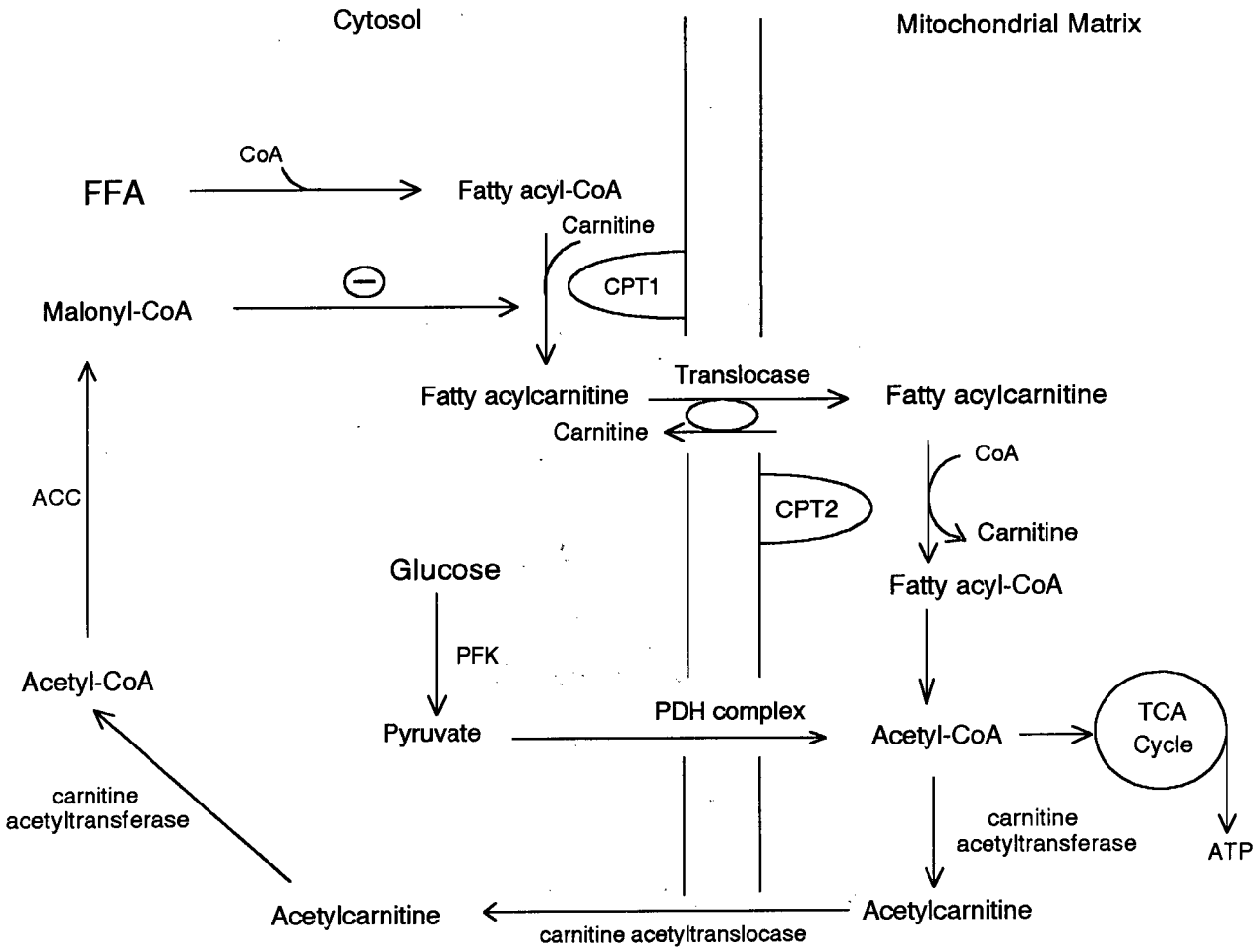


Figure 1. Cardiac Fatty Acids and Glucose Metabolism.

(Modified from Rodrigues and McNeill 1992; Lopaschuk and Gamble 1994)

FFAs are transported into cardiac myocytes either by saturable, carrier-mediated transporters (fatty acid binding proteins, FABPs) or by nonsaturable diffusion across the plasma membrane (Schulz 1991). In the cytosol, fatty acids can be used for intracellular synthesis of complex lipids or they can enter the mitochondria and undergo β oxidation. To undergo β oxidation, the long-chain fatty acids must first be activated by CoA to form fatty acyl CoA on the outer surface of the inner mitochondrial membrane and in the presence of ATP, Mg^{2+}, and thiokinase. As the inner mitochondrial membrane is impermeable to fatty acyl CoAs, the fatty acyl groups must be transferred from CoA to carnitine in the presence of carnitine palmitoyl transferase 1 (CPT1). The fatty acyl carnitine formed is allowed to enter the mitochondrial matrix by a specific translocase in exchange for acetyl carnitine or free carnitine, and is reconverted to fatty acyl CoA by carnitine palmitoyl transferase 2 (CPT2) which is located on the inner surface of the matrix membrane. Through β oxidation, the acyl CoA within the mitochondrial matrix produces acetyl CoA which enters the tricarboxylic acid (TCA) cycle for final oxidation and ATP production.

In cardiac myocytes, glucose cannot pass through the lipid bilayer freely. It is transported by specific integral membrane proteins down a concentration gradient. In the cardiac cells, glucose disposal occurs either through an oxidative pathway resulting in complete degradation of glucose, or a non-oxidative pathway leading to glycogen synthesis (DeFronzo *et al.* 1981) and glycolysis. Glucose is first irreversibly phosphorylated to glucose-6-phosphate (G-6-P) by hexokinase. The G-6-P may either be converted to or formed from glycogen, or broken down to pyruvate by phosphofructokinase (PFK), which is a rate limiting enzyme in glycolysis. The pyruvate enters the mitochondria and is converted irreversibly to acetyl CoA by the pyruvate dehydrogenase (PDH) complex, which is located

exclusively in the mitochondrial compartment and is present in high concentrations in cardiac myocytes. The acetyl CoA formed then enters the TCA cycle for complete oxidation and ATP formation.

Several enzymes in the glucose oxidation pathway such as PFK, hexokinase, glyceraldehyde phosphate dehydrogenase and PDH are inhibited by concurrent fatty acid oxidation. Hence fatty acids are the main energy source of the myocardium, and are preferentially utilized when available (Shipp *et al.* 1961). Glucose oxidation can also regulate fatty acid oxidation through acetyl-CoA carboxylase (ACC) (Lopaschuk and Gamble 1994). An increase in the intramitochondrial acetyl-CoA levels which is secondary to activation of PDH or a decrease in demand for acetyl-CoA by the TCA cycle activates carnitine acetyltransferase, which transfers acetyl groups from acetyl-CoA to acetylcarnitine. Acetylcarnitine is transported into the aqueous cytoplasm by a carnitine translocase. The acetyl groups are then transferred back onto acetyl-CoA. An increase in aqueous cytoplasmic acetyl-CoA activates a 280-kDa isoform of ACC, which is a potential source of malonyl-CoA. Malonyl-CoA, a potent inhibitor of CPT1, prevents fatty acids from entering the mitochondria to undergo oxidation. Therefore, ACC has an important role in regulating the balance between carbohydrate and lipid metabolism in the heart. At a constant workload and TCA cycle activity, an increase in acetyl-CoA derived from glucose oxidation is matched by a parallel decrease in acetyl-CoA supply from β-oxidation of fatty acids.

During diabetes, there is a marked increase in adipose tissue lipolysis with a subsequent outflow of FFAs (Rodrigues *et al.* 1995). The FABPs content of the diabetic heart is also markedly increased (Glatz *et al.* 1994) resulting in high levels of cytosolic fatty acids. This is usually associated with a reduced cardiac contractile

force and greater cardiac susceptibility to arrhythmias (Opie 1970). High levels of cytosolic fatty acids also result in an increase in the oxidation of fatty acids by the diabetic hearts. The consequence is a high requirement for oxygen and an intracellular accumulation of toxic fatty acid metabolic intermediates, such as long chain acyl-CoA and acyl carnitine. In diabetic hearts, the increased fatty acid oxidation may also be due to a decrease in ACC activity (Lopaschuk and Gamble 1994).

Glucose oxidation is markedly depressed in the diabetic heart, probably as a result of the following:

i) Impaired glucose transport into the cardiac cells:

A major metabolic action of insulin is to stimulate glucose entry and hence oxidation. This effect of controlling glucose transport is achieved by inducing a rapid reversible translocation of glucose transporter proteins (GLUT-4) from a latent intracellular pool to the plasma membrane (Suzuki and Kono 1980; Cushman and Wardzala 1980; Karnieli *et al.* 1981). Activation of glucose transport by insulin leads to enhanced glycolytic rates and glycogen deposition which would contribute in large part to the hypoglycemic action of insulin *in vivo*. In diabetic rats, the insulin sensitive GLUT-4 protein and mRNA are specifically reduced in cardiomyocytes (Eckel and Reinauer 1990). This restricts glucose transport across the sarcolemmal membrane into the myocardium (Kobayashi and Olefsky 1979; Karnieli *et al.* 1987; Garvey *et al.* 1989), and hence impairs glucose utilization.

ii) Reduced rate of glucose phosphorylation:

Randle and colleagues (1963) have suggested that the increased availability of free fatty acids can increase the TCA cycle activity and thus the citrate concentration. The citrate formed may inhibit phosphofructokinase, thereby decreasing the rate of glycolysis and this in turn results in an impaired glucose uptake and oxidation. It has been shown that the elevated FFAs decrease glucose uptake into the heart by approximately 30% (Nuutila *et al.* 1992). The reduction in substrate flow through the glycolytic pathway results in an eventual increase in the tissue levels of glucose-6-phosphate, which then activates glycogen synthesis and inhibits glycogen phosphorylase activity (Chen and Lanuzzo 1982). Hence cardiac glycogen content is increased by 3-4 fold in diabetic heart (Chen and Lanuzzo 1982).

iii) Reduced pyruvate dehydrogenase activity:

Pyruvate dehydrogenase activity is depressed in diabetes (Wieland *et al.* 1971) leading to an impaired pyruvate oxidation in the heart. This depression is again a likely result of increased fatty acid oxidation. Increased fatty acid oxidation leads to an increased acetyl CoA/CoA ratio and this activates the PDH kinase to phosphorylate and inactivate the PDH complex. PDH kinase activity has been shown to be elevated in diabetic hearts, accounting for the decrease in the active form of PDH (Hutson *et al.* 1978). Perfusion of the non-diabetic rat heart with fatty acids also leads to an increase in tissue acetyl CoA/CoA ratio and a decrease in pyruvate oxidation and PDH complex activity (Latipaa *et al.* 1985). Furthermore, fatty acids inhibit glucose oxidation to a greater extent at the level of pyruvate dehydrogenase than at the level of phosphofructokinase (Saddik and Lopaschuk

1991), as in the presence of high concentrations of fatty acids, rates of glycolysis are more than 13 times the rates of glucose oxidation. Thus, in the presence of high concentrations of free fatty acids, myocardial glucose oxidation is essentially abolished in chronically diabetic rats.

4. Glucose oxidation and heart function

Glucose oxidation in the heart is important as: 1) glycolytically produced ATP could be preferentially used by membrane ion pumps such as sarcolemmal ATPase (Bunger *et al.* 1986). 2) Increasing flux through the pyruvate dehydrogenase complex will prevent the accumulation of glycolytic products such as lactate, which may accumulate under ischemic conditions. 3) Glucose consumes less myocardial oxygen for any given level of cardiac work. It has been shown that complete oxidation of glucose produces 3.17 mol ATP/mol O_2, whereas palmitate only produces 2.8 mol ATP/mol O_2.

Rosen *et al.* (1986) have indicated that in chronically diabetic rat hearts, diminished glucose conversion and depressed energy production from glucose are associated with an impaired *in vivo* cardiac performance. Given that excessive oxidation of fatty acids is responsible for the depressed glucose oxidation and that glucose oxidation is beneficial for the heart, a switch from predominantly fatty acids to carbohydrate oxidation could be thought to have salutary effects on the diabetic heart. This has been achieved by inhibition of fatty acid oxidation and/or stimulation of glucose oxidation.

Normal cardiomyocytes cultured in the presence of palmitate exhibit a markedly depressed insulin-stimulated glucose oxidation which is partially restored

by inhibition of fatty acid oxidation (Eckel *et al.* 1991). Selective inhibition of carnitine palmitoyltransferase 1 (CPT1) by etomoxir restored the glucose oxidation and improved diabetic heart function (Rosen and Reinauer 1984). Perfusion of the STZ-diabetic rat heart by phenylalkyloxiranecarboxylic acid (POCA), a selective CPT1 inhibitor, reduced myocardial lipolysis and accelerated glycolysis and glucose oxidation (Rosen and Reinauer 1984).

Perfusion of the diabetic heart with dichloroacetate (DCA), a glucose oxidation stimulator, acutely reversed the depressed cardiac function seen in chronically diabetic rats (Nicholl *et al.* 1991). DCA is well known as a protein kinase inhibitor involved in the regulation of the PDH complex. The stimulating effect of DCA on the PDH complex is via inhibition of pyruvate dehydrogenase kinase (Stacpoole and Greene 1992). Hydralazine (Rodrigues *et al.* 1986) and carnitine (Rodrigues *et al.* 1988) which improve diabetic cardiac function have been shown to stimulate glucose oxidation in fatty acid perfused hearts (Burns *et al.* 1991; Broderick *et al.* 1992).

5. Effect of vanadium on glucose oxidation

It is well established that chronic treatment of STZ-diabetic rats with vanadium results in euglycemia and an improved cardiac function (Heyliger *et al.* 1985; Meyerovitch *et al.* 1987; Ramanadham *et al.* 1989). In skeletal muscle, vanadate increases glucose uptake, glycogen synthesis, glycolysis and glucose oxidation (Clark *et al.* 1985). In isolated rat adipocytes, vanadate stimulated glucose oxidation in a manner similar to insulin (Dubyak and Kleinzeller 1980; Duckworth *et al.* 1988). Vanadate has also been shown to potentiate insulin-stimulated glucose oxidation in isolated cardiomyocytes (Rodrigues *et al.* 1995).

BMOV is a new organic form of vanadium (McNeill *et al.* 1992) which has been shown to be more potent and less toxic than the inorganic form of vanadium in reverting hyperglycemia and improving cardiac function in chronically STZ-diabetic rats (Yuen *et al.* 1993a,b). However, the mechanism of this beneficial effect of BMOV on heart function has yet to be determined.

Hypothesis:

BMOV treatment of diabetic rats *in vivo* improves cardiac function by enhancing glucose oxidation in the heart.

Rationale:

Fatty acids are the principle source of energy utilized by the normal heart. In diabetic hearts, there is an excessive oxidation of free fatty acid and/or an impaired glucose oxidation, which occurs concomitantly with a depressed cardiac function. Insulin improves diabetic heart function and increases glucose oxidation. The insulin mimetic vanadate, also improves heart function and potentiates insulin stimulated glucose oxidation. BMOV is an organic form of vanadium and has been shown to be more potent than inorganic form of vanadium. Therefore, the improved heart function after BMOV treatment may be accompanied or caused by an enhanced glucose oxidation in the heart.

Objectives:

To study the effect of BMOV on glucose oxidation in cardiac myocytes from control and acutely STZ-diabetic rats. We hence measured:

1) Basal glucose oxidation in cardiac myocytes isolated from control and acutely STZ-diabetic rats.
2) Effect of insulin on glucose oxidation in cardiac myocytes isolated from control and acutely STZ-diabetic rats.

3) Effect of BMOV on glucose oxidation in cardiac myocytes isolated from control and STZ-diabetic rats.
4) Effect of BMOV on insulin-stimulated glucose oxidation in cardiac myocytes isolated from control and STZ-diabetic rats.

MATERIALS AND METHODS

1. Materials

1.1. Animals

Male Wistar rats weighing 260-280 g were obtained from Animal Care Center, University of British Columbia, Vancouver, B.C., Canada. They were maintained on a 12 hour light/dark cycle and were allowed free access to standard laboratory food (Purina rat chow) and water.

1.2. Chemicals

BDH, Vancouver, B.C., Canada

Potassium hydroxide, sulphuric acid, D-glucose anhydrous, calcium chloride dihydrate, sodium chloride and hydrochloric acid.

Sigma Chemical Co., St. Louis, MO, U.S.A.

Streptozotocin, magnesium sulphate, L-carnitine, sodium bicarbonate and insulin.

Life Technologies, Grand Island, NJ, U.S.A.

Joklik minimum essential medium with 11 mM glucose.

Stemcell Technologies Inc., Vancouver, B.C., Canada

Joklik minimum essential medium without glucose.

Worthington Biochemical Corporation, NJ, U.S.A.

Collagenase (325 U/mg).

Boehringer Mannheim GmbH, Germany

Albumin, Fraktion V.

Fisher Scientific, Fair Lawn, NJ, U.S.A.

ScintiVerse.

Do Pont Canada Inc., Ontario, Canada

^{14}C-glucose.

2. Methods

2.1. Induction of diabetes

Diabetes was induced by a single tail vein injection of 100 mg/kg STZ which was dissolved in 0.9% NaCl solution. Control rats were injected with vehicle alone. Both injections were made under light halothane anesthesia. Myocytes were prepared from control and diabetic rats after 3-7 days of STZ injection. The acute diabetic state was confirmed by the presence of hyperglycemia (>20 mM) in blood samples obtained from tail vein, using Glucostix reagent strips read by a Glucometer II.

2.2. Isolation of cardiac myocytes

The Ca^{2+}-tolerant cardiac myocytes were isolated as described by Rodrigues *et al.* (1992). The rat was deeply anesthetized by an i.p. injection 65 mg/kg pentobarbital, and blood was prevented from clotting by an i.p. injection of 0.8 mL heparin (1000 U/mL). The heart was quickly excised and perfused in the Langendorff mode under 95% O_2 / 5% CO_2. The perfusate was Joklik MEM containing 11 mM glucose, 24 mM $NaHCO_3$, 1.2 mM $MgSO_4$, and 1.2 mM L-carnitine. After 5 minutes, the perfusate was switched to Joklik containing 195 U/mL collagenase, 25 μM Ca^{2+} and 1 mg/mL bovine serum albumin. The heart was perfused for 25-30 minutes at a flow-rate of 6-7 mL/min. Compared to control hearts, STZ-diabetic rat hearts were more sensitive to collagenase digestion and had a shorter digestion time (15-20 minutes). After becoming flaccid, the heart was cut just below the atrium. The ventricle tissues were cut into strips and further separated by vigorous shaking. After complete digestion, the cells were filtered through 200 μ mesh and centrifuged for 1 minute at 45×g. The pellet was resuspended in Joklik containing increasing concentrations of Ca^{2+} (0.2, 0.5 and 1 mM) and the cells were counted using a microscope.

2.3. Determination of cell viability and yield

Cell viability was examined by the Trypan blue exclusion method. 0.4% Trypan Blue (20 μl) was added to the cells (20 μl) in a small test tube. This mixture (9 μl) was put under the cover slip of a microscope. Viable cells were able to exclude Trypan Blue and were rod shaped. Dead cells could not exclude Trypan Blue and were round in shape. Cell viability and yield were determined by counting cells in two sets of 16 squares in a hemocytometer.

Cell viability = Number of live cells in 2 squares / Total number of cells in 2 squares

Yield = Total number of cells in 2 squares × Total volume of cell suspension × 10^4

2.4. Determination of glucose oxidation in cardiac myocytes

All the samples were analyzed in triplicate. Cardiac myocytes were transferred into Joklik-MEM containing 0.2 mM glucose. 0.4×10^6 cells were incubated with 1 μCi/mL D-[U-^{14}C]-glucose at 37°C and under 95% O_2 / 5% CO_2 for 60 minutes. At 55 minutes, rubber stoppers with a center well containing rolled filter paper (1×4 cm^2) saturated with 200 μl of 5% KOH were inserted over the tubes. At 60 minutes, 250 μl of 1N H_2SO_4 was added to the cells to stop the reaction and convert $[H^{14}CO_3]^-$ in the medium to $^{14}CO_2$ which was trapped by the filter paper. The filter paper was then put into scintillation vials in which 10 mL scintillation fluid was added. The vials were shaken at room temperature for 24 hours and the radioactivity was counted.

2.5. Insulin dose response curves

0.4×10^6 cells isolated from control or acutely STZ-diabetic rat hearts were incubated with 1 μCi/mL of D-[U-^{14}C]-glucose at 37°C for 60 minutes in the presence of 0, 25, 50, 100, 200 and 500 ng/mL insulin. The glucose oxidation was measured as described above.

2.6. BMOV dose response curves (± insulin)

Cardiac myocytes isolated from control or acutely STZ-diabetic rats were preincubated at 37°C with 0, 10, 50, 100 and 500 μM BMOV in Joklik containing 11

mM glucose for 30 minutes. The myocytes were centrifuged and resuspended in Joklik containing 0.2 mM glucose. Then 0.4×10^6 myocytes were incubated with labelled D-[U-^{14}C]-glucose in the presence or absence of 100 ng/mL insulin at 37° C for 60 minutes. The glucose oxidation was measured as described above.

RESULTS

1. Effect of diabetes on glucose oxidation

Glucose oxidation was greatly affected by diabetic state (Fig. 1). Basal glucose oxidation was significantly reduced in diabetic myocytes compared to the basal level of control myocytes. Insulin, at the concentration of 100 ng/mL, significantly increased glucose oxidation in cardiac myocytes isolated from both control and STZ-diabetic rats. However, the increased glucose oxidation in diabetic myocytes was still lower than the basal level in control myocytes.

2. Insulin dose response curves in cardiac myocytes isolated from control and acutely STZ-diabetic rats

The insulin dose response curves in cardiac myocytes isolated from control and acutely STZ-diabetic rats are shown in Fig. 2. Insulin at doses of 50, 100, 200, and 500 ng/mL significantly increased glucose oxidation in both control and diabetic myocytes. The insulin-stimulated glucose oxidation was not significantly different among these insulin concentrations. However, the response was significantly less in diabetic myocytes than in control myocytes.

3. Effect of BMOV on glucose oxidation in cardiac myocytes from control rats

In cardiac myocytes isolated from control rats, preincubation with BMOV within the range of 50, 100 and 500 μM for 30 minutes did not enhance glucose oxidation. Additionally, BMOV did not further enhance insulin-stimulated glucose oxidation (Fig. 3).

4. Effect of BMOV on glucose oxidation in cardiac myocytes isolated from acutely STZ-diabetic rats

In cardiac myocytes isolated from acutely STZ-diabetic rats, preincubation with BMOV at concentrations of 50, 100, 500 μM significantly increased glucose oxidation when compared to basal (Fig. 4). The increased glucose oxidation was not different among these three BMOV concentrations.

Since there was no difference in the effect of BMOV on glucose oxidation over the range of 50-500 μM, the values were combined. The increased glucose oxidation by BMOV was equivalent to that produced by the maximally stimulating concentration of insulin (100 ng/mL). Furthermore, BMOV within the range of 50-500 μM significantly enhanced insulin-stimulated glucose oxidation (Fig. 5).

Figure 1

Effect of insulin on glucose oxidation in cardiac myocytes isolated from control and acutely STZ-diabetic rats. Ca^{2+}-tolerant myocytes were isolated from control (n=10) and diabetic (100 mg/kg STZ, 3 days, n=7) rat hearts as previously described by Rodrigues *et al.* 1992. Glucose oxidation was initiated by incubating myocytes with 1 μCi/mL D-[U-^{14}C] glucose for 60 minutes in the presence and absence of insulin (100 ng/mL). The amount of $^{14}CO_2$ produced was measured.

Results are expressed as mean ± S.E.M.

* denotes significantly different from the basal level of control rats.

# denotes significantly different from the basal level of diabetic rats; $p< 0.05$.

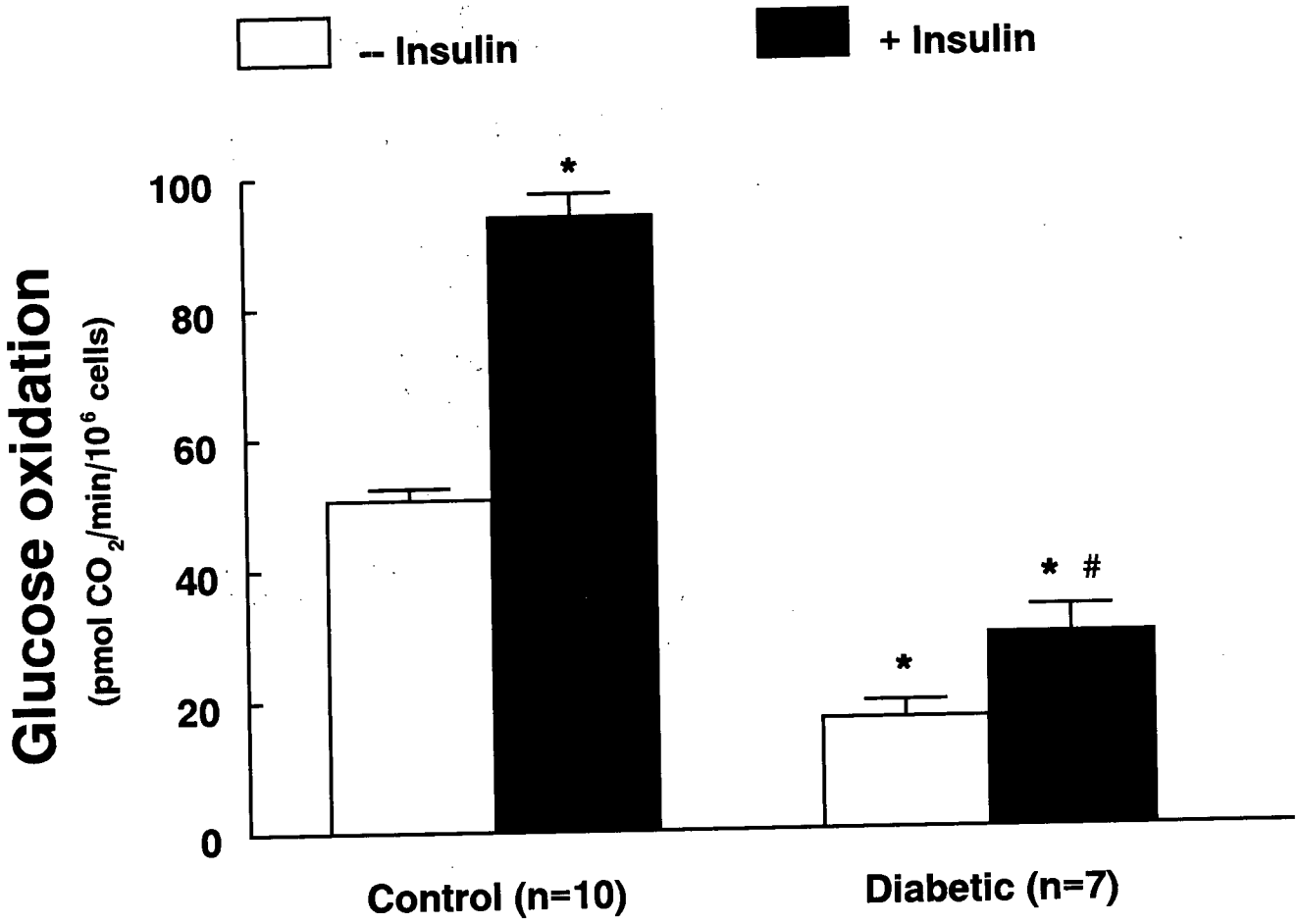

-- Insulin
+ Insulin
Glucose oxidation
(pmol CO_2/min/10^6 cells)
100
80
60
40
20
0
*
*
* #
Control (n=10)
Diabetic (n=7)

Figure 2

Insulin dose-response curves in cardiac myocytes isolated from control and acutely STZ-diabetic rats. Cardiac myocytes isolated from control (n=8) and diabetic (100 mg/kg, 3 days, n=6) rat hearts were incubated with 1 μCi/mL D-[U-^{14}C] glucose and 0, 25, 50, 100, 200 and 500 ng/mL insulin for 60 minutes at 37°C. The amount of $^{14}CO_2$ produced was measured.

Results are expressed as mean ± S.E.M.

* denotes significantly different from the basal value of control rats.

# denotes significantly different from the basal value of diabetic rats; $p< 0.05$.

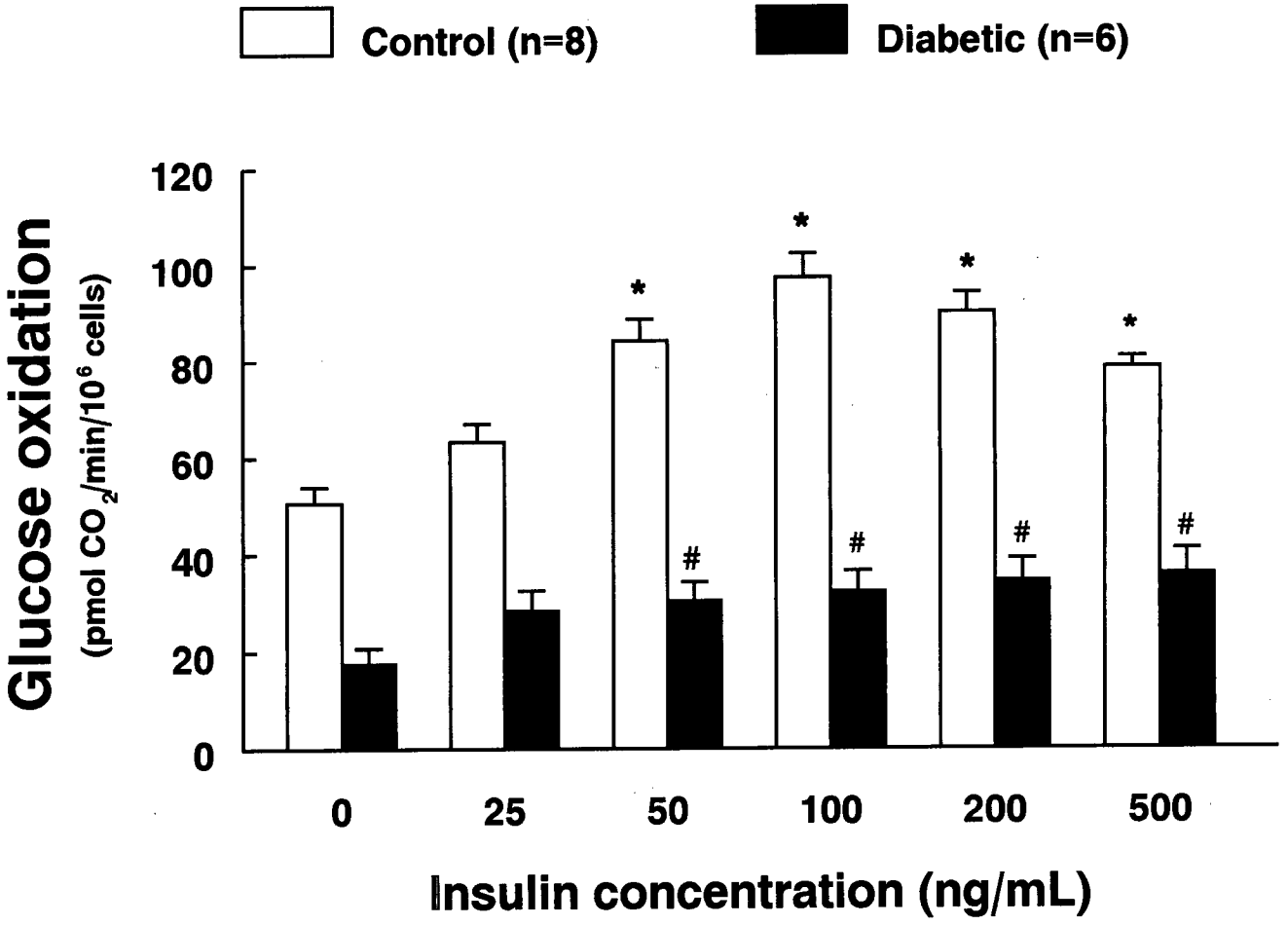

Control (n=8)
Diabetic (n=6)
Glucose oxidation
(pmol CO2/min/10^6 cells)
120
100
80
60
40
20
0
*
#
0
25
50
100
200
500
Insulin concentration (ng/mL)

Figure 3

Effect of BMOV on glucose oxidation in cardiac myocytes isolated from control rats. Cardiac myocytes isolated from control rat hearts (n=8) were incubated with 1 μ Ci/mL D-[U-^{14}C] glucose (±100 ng/mL insulin) for 60 min, after preincubation with BMOV (50-500 μM) for 30 min. The amount of $^{14}CO_2$ produced was measured.

Results are expressed as mean ± S.E.M.

* denotes significantly different from the basal level of control rats; $p< 0.05$.

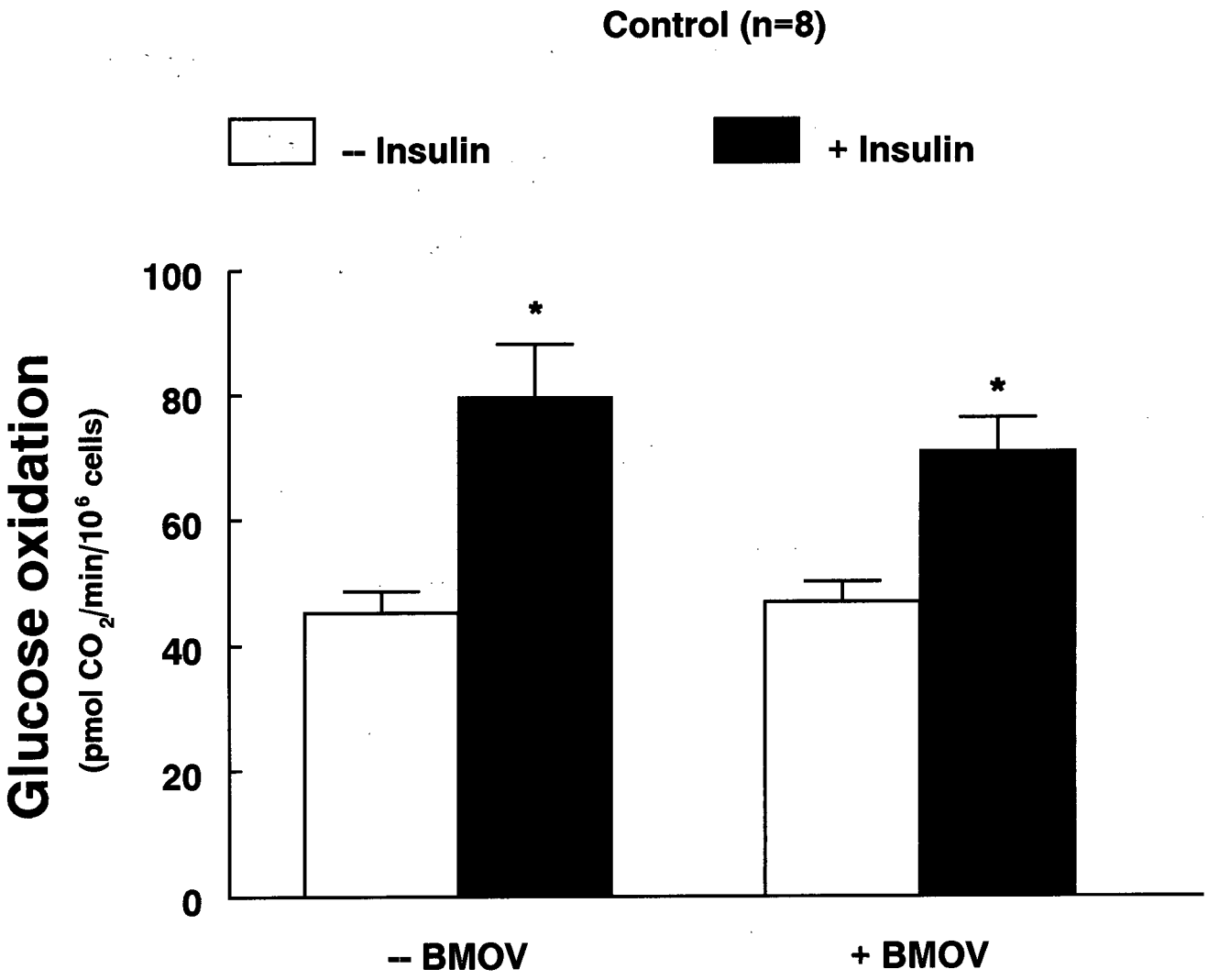

Control (n=8)
-- Insulin
+ Insulin
Glucose oxidation
(pmol CO2/min/10^6 cells)
100
80
60
40
20
0
*
*
-- BMOV
+ BMOV

Figure 4

Effect of BMOV on glucose oxidation in cardiac myocytes isolated from acutely STZ-diabetic rats. Cardiac myocytes isolated from diabetic rat hearts (100 mg/kg STZ, 3 days, n=6) were preincubated with 1 μCi/mL D-[U-^{14}C] glucose for 60 min, after preincubation with BMOV at concentrations of 0, 10, 50, 100, and 500 μM for 30 min. The amount of $^{14}CO_2$ produced was measured.

Results are expressed as mean ± S.E.M.

* denotes significantly different from the basal level of diabetic rats; $p < 0.05$.

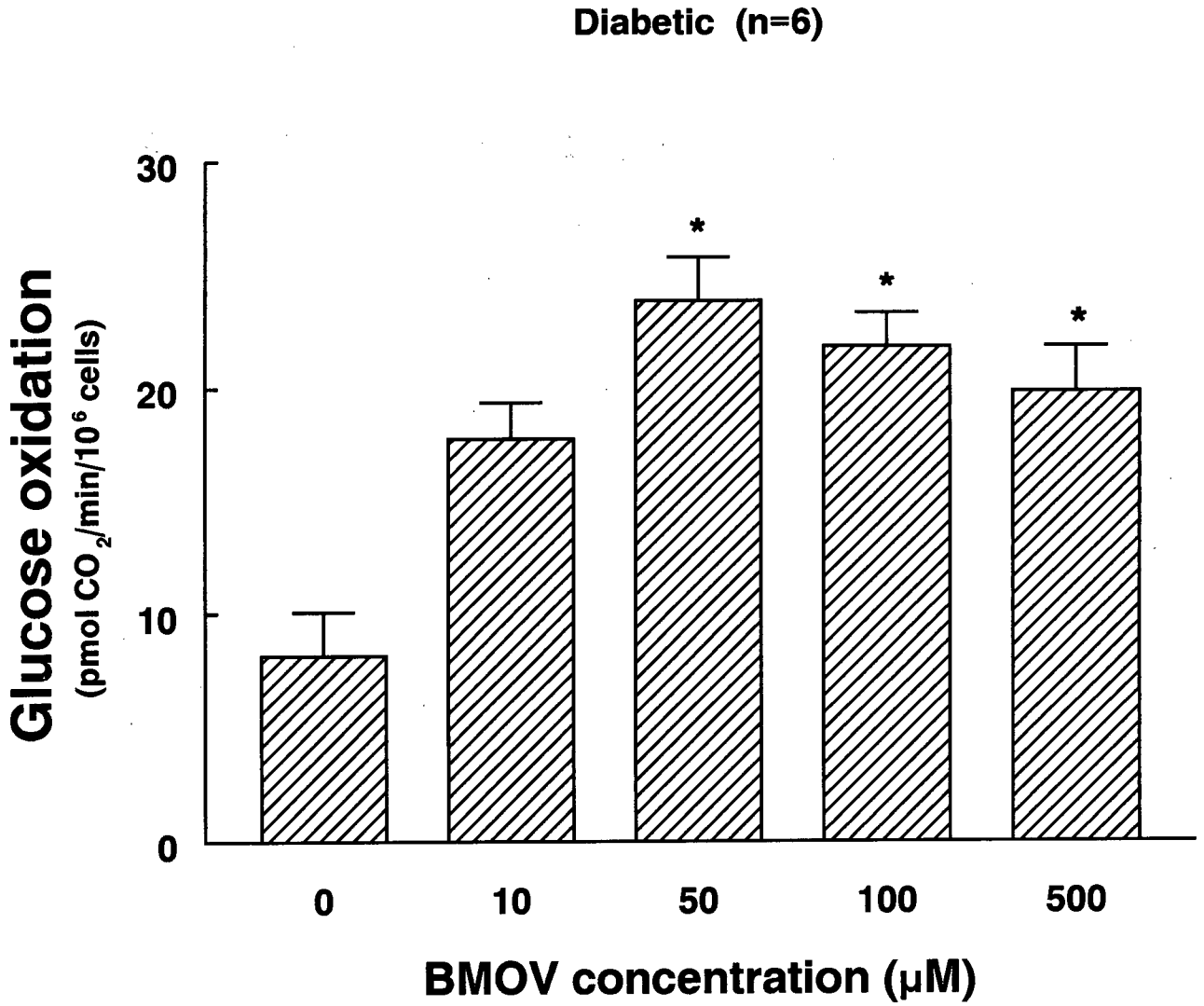

Diabetic (n=6)
Glucose oxidation
(pmol CO2/min/10^6 cells)
30
20
10
0
*
*
*
0
10
50
100
500
BMOV concentration (μM)

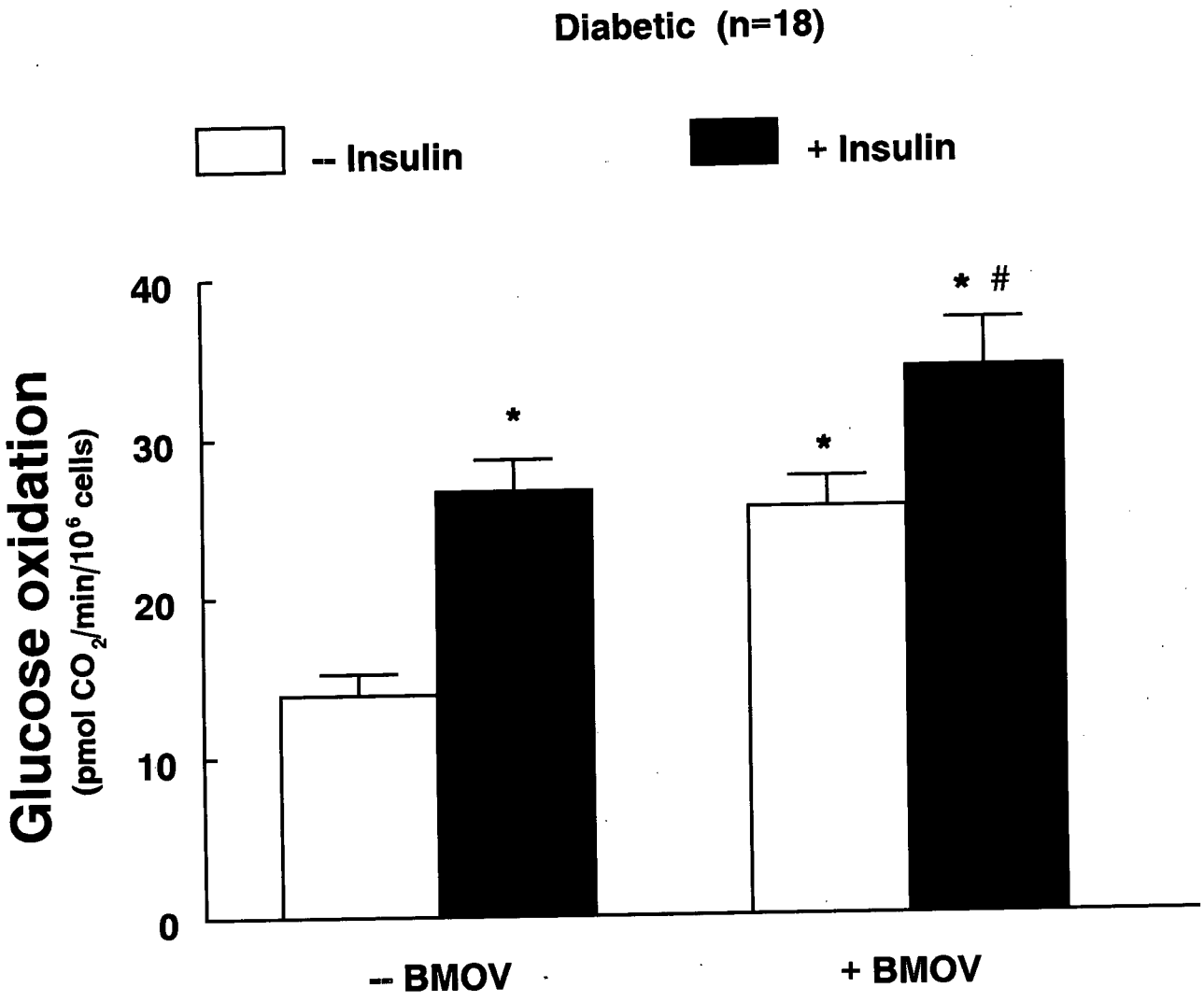
Figure 5

Effect of BMOV on insulin-stimulated glucose oxidation in cardiac myocytes isolated from acutely STZ-diabetic rats. Cardiac myocytes isolated from diabetic rat hearts (100 mg/kg, 3 days, n=18) were incubated with 1 μCi/mL D-[U-^{14}C] glucose (±100 ng/mL insulin) for 60 min, after preincubation with BMOV (50-500 μM) for 30 min. The amount of $^{14}CO_2$ produced was measured.

Results are expressed as mean ± S.E.M.

* denotes significantly different from the basal level of control rats.

# denotes significantly different from insulin-stimulated glucose oxidation; $p< 0.05$.


Diabetic (n=18)
-- Insulin
+ Insulin
Glucose oxidation
(pmol CO_2/min/10^6 cells)
40
30
20
10
0
*
*
* #
-- BMOV
+ BMOV

DISCUSSION

Under normal conditions, the heart derives about 70% of its energy from fatty acids and about 30% from glucose (Neely and Morgan 1974). In diabetic heart, as a result of elevation of circulating fatty acids, glucose utilization is essentially abolished and fatty acids can account for up to 99% of the oxidative fuel of cardiac muscle (Garland *et al.* 1962). Increased fatty acid oxidation and depressed glucose oxidation rates can be demonstrated in isolated working heart models (Wall and Lopaschuk 1989) and in isolated cardiac myocytes from STZ-diabetic rats (Chen *et al.* 1984; Rodrigues *et al.* 1995). Depressed glucose oxidation is reported to occur concomitantly with a depressed heart function (Chatham and Forder 1993). Thus, it has been proposed that metabolic derangements may play an important role in the pathogenesis of diabetic cardiomyopathy. A switch from fatty acid oxidation to glucose oxidation may revert the metabolic derangement and improve heart function. An enhancement of glucose oxidation by BMOV in isolated myocytes may provide an explanation for the improvement in cardiac function previously noted in *in vivo* studies.

In isolated rat adipocytes, vanadate elicited an insulin-like stimulation of glucose oxidation that was dependent on extracellular vanadate concentrations, with a maximal stimulation at 10 mM (Dubyak and Kleinzeller 1980). In isolated diabetic cardiomyocytes, vanadate at the concentration of 1 mM potentiated insulin-stimulated glucose oxidation (Rodrigues *et al.* 1995). In the present study, BMOV at the concentrations of 0.05-0.5 mM stimulated glucose oxidation and further enhanced insulin-stimulated glucose oxidation. This is consistent with previous results from *in vivo* studies which demonstrated that the organic form of vanadium BMOV is more potent than inorganic form of vanadate. It should be noted that the

vanadate stimulated glucose oxidation in adipocytes was noted when adipocytes were incubated with both vanadate and ^{14}C-glucose simultaneously. However, the increased glucose oxidation in cardiac myocytes can only be seen after preincubation with vanadate for 60 minutes (Rodrigues *et al.* 1995) or preincubation with BMOV for 30 minutes. The reason for the discrepancy between adipocytes and cardiac myocytes in their responses to vanadium is not clear.

In isolated cardiac myocytes, the maximal stimulation of glucose oxidation in BMOV pretreated myocytes is equivalent to that produced by a maximally stimulating concentration of insulin. In addition, preincubation of the cells with BMOV within the range of 50-500 μM enhances insulin stimulated-glucose oxidation. Glucose oxidation is only increased when myocytes are pretreated with BMOV, while insulin did not need preincubation. This may suggest that BMOV and insulin exert their effects on glucose oxidation through different mechanisms.

In diabetic hearts, the depressed glucose oxidation is increased by BMOV. BMOV may act at several steps to increase glucose oxidation. These include: 1) Stimulation of glucose transporters on the cardiac sarcolemmal membrane to increase glucose uptake and utilization; 2) Inhibition of fatty acid oxidation by selective inhibition of CPT1 activity, an action similar to etomoxir; 3) Direct stimulation of the PDH complex activity, an action similar to DCA; 4) Inhibition of endogenous triglyceride lipolysis by inhibition of triglyceride lipase. Endogenous mobilizable myocardial triglycerides are an important source of fatty acids for oxidative metabolism (Kryski *et al.* 1985) and their contribution to overall energy production ranges from 11% of myocardial ATP requirements in hearts perfused with high fat to more than 50% when the heart is deprived of exogenous fatty acids (Saddik and Lopaschuk 1991). In diabetic heart, myocardial triglyceride content

increases (Denton and Randle 1967; Murthy and Shipp 1977; Rizza *et al.* 1971). Oxidation of endogenous triglyceride now accounts for 70% of overall myocardial ATP requirement (Saddik and Lopaschuk 1994). Therefore, an inhibition of endogenous triglyceride lipolysis by BMOV may contribute to the increased glucose oxidation in the diabetic hearts and 5) Enhancing ACC activity to increase the production of malonyl-CoA which is a potential CPT1 inhibitor. Therefore, an increased ACC activity by BMOV and the resultant increase in malonyl-CoA levels may cause an inhibition of fatty acid oxidation leading to an increase in glucose oxidation.

In this study, insulin increased glucose oxidation in cardiac myocytes from control and acutely STZ-diabetic rats by approximately 2-fold. This result is consistent with previous studies in which insulin increased glucose oxidation by 2-fold in isolated perfused rat hearts (Rosen *et al.* 1984; 1986), isolated cardiac myocytes (Rodrigues *et al.* 1995) and cultured cardiac myocytes (Claycomb *et al.* 1984) even though insulin increased glucose uptake by 8-20 fold (Fischer *et al.* 1991) and PDH complex activity by 3.5-fold in the heart (Cooney *et al.* 1993). Similarly, insulin increased glucose oxidation by 2-fold in STZ-diabetic rat hearts (Rosen *et al.* 1986) and in STZ-diabetic cardiomyocytes (Rodrigues *et al.* 1995). Compared to other tissues, insulin increased glucose oxidation by 1-4 fold in isolated adipocytes (Kobayashi and Olefsky 1979; Shechter and Karlish 1980; Murer *et al.* 1992) and by 2-fold in skeletal muscles (Clark *et al.* 1985).

The quiescent cardiac myocytes isolated in this study responded to insulin stimulation similarly to perfused beating heart. This may indicate that myocytes isolated by this method possess the same characteristics as whole heart and can be used to study the direct effect of drugs on the heart. However, the increased

glucose oxidation by insulin can only be seen at concentrations greater than physiological levels (> 50 ng/mL).

The advantages of using myocytes are: 1) They are fully differentiated and morphologically similar to cells in the intact heart, but lack interstitial tissues and contamination from other cell types which can complicate measurement in whole tissues (Rodrigues *et al.* 1995); 2) Direct effect of a drug on the heart functional units can be observed; and 3) Effect of circulating lipids can be removed. The disadvantages of using myocytes are: 1) The metabolic fate of substrate could be different in the non-beating myocytes as compared to beating hearts (Rodrigues *et al.* 1995) and 2) Isolated cardiac myocytes can be kept alive only for a limited period of time, which may limit the experimental conditions.

In summary, depressed glucose utilization in diabetic hearts may contribute to the pathogenesis of diabetic cardiomyopathy. The enhanced glucose oxidation in cardiac myocytes by BMOV may contribute to the improved cardiac function in STZ-diabetic rats.

CONCLUSIONS

1. Glucose oxidation was depressed in cardiac myocytes isolated from acutely STZ-diabetic rats (100 mg/kg, i.v., 3-7 days) compared to cells isolated from control rats.

2. Insulin increased glucose oxidation in cardiac myocytes isolated from both control and acutely STZ-diabetic rats. However, the increased glucose oxidation in diabetic myocytes was still lower than control value.

3. In isolated cardiac myocytes isolated from control rats, BMOV had no effect on glucose oxidation and did not enhance insulin-stimulated glucose oxidation.

4. In cardiac myocytes isolated from acutely STZ-diabetic rats, BMOV within the range of 50-500 μM caused an increase in glucose oxidation. The increased glucose oxidation caused by BMOV was equivalent to that produced by the maximally stimulating concentration of insulin. In addition, BMOV enhanced insulin-stimulated glucose oxidation.

REFERENCES

Ar'Rajab, A. and Ahren, B. (1993). Long-term diabetogenic effect of streptozotocin in rats. Pancreas 8 (1): 50-57.

Battell, M.L., Yuen, V.G. and McNeill, J.H. (1992). Treatment of BB rats with vanadyl sulphate. Pharmacol. Commun. 1: 291-301.

Bollen, M., Miralpeix, M., Ventura, F., Toth, B., Bartrons, R. and Stalmans, W. (1990). Oral administration of vanadate to streptozotocin-diabetic rats restores the glucose-induced activation of liver glycogen synthase. Biochem. J. 267: 269-271.

Brichard. S.M., Pottier, A.M. and Henquin, J.C. (1989). Long term improvement of glucose homeostasis by vanadate in obese hyperinsulinemic fa/fa rats. Endocrinology 125: 2510-2516.

Broderick , T.L., Quinney, H.A. and Lopaschuk, G.D. (1992). Carnitine stimulation of glucose oxidation in the fatty acid perfused isolated working heart. J. Biol. Chem. 267: 3758-3763.

Bunger, R., Swindall, B., Brodie, D., Zdunek, D., Stiegler, H. and Walter, G. (1986). Pyruvate attenuation of hypoxia damage in isolated working guinea-pig heart. J. Mol. Cell. Cardiol. 18: 423-428, 156.

Burns, A.H., Burns, L.A.R., Jurenka, L.U. and Summer, W.R. (1991). Myocardial metabolic effects of in vivo hydralazine treatment of streptozotocin-diabetic rats. Am. J. Physiol. 260: 516-521.

Cam, M.C, Cros, G.H., Serrano, J.J., Lazaro, R. and McNeill, J.H. (1993). Long-term effectiveness of oral vanadyl sulphate in streptozotocin-diabetic rats. Diabetologia 36: 218-224.

Cam, M.C., Faun, J. and McNeill, J.H. (1995). Concentration-dependent glucose-lowering effects of oral vanadyl are maintained following treatment withdrawal in streptozotocin-diabetic rats. Metabolism 44 (3): 332-339.

Chatham, J.C. and Forder, J.R. (1993). A ^{13}C-NMR study of glucose oxidation in the intact functional rat heart following diabetes-induced cardiomyopathy. J. Mol. Cell. Cardiol. 25: 1203-1213.

Chen, V. and Lanuzzo, C.D. (1982). Dosage effect of streptozotocin on rat tissue enzyme activities and glycogen concentration. Can. J. Physiol. Pharmacol. 60: 1251-1256.

Chen, V., Lanuzzo, C.D. Fong. B.C. and Spitzer, J.J. (1984). The effects of acute and chronic diabetes on myocardial metabolism in rats. Diabetes 33: 1078-1084.

Clark, A.S., Fagan, J.M. and Mitch, W.E. (1985). Selectivity of the insulin-like actions of vanadyl on glucose and protein metabolism in skeletal muscle. Biochem. J. 232: 272-276.

Claycomb, W.C., Burns, A.H. and Shepherd, R.E. (1984). Culture of the terminally differentiated ventricular cardiac muscle cell. FEBS Letters 169 (2). 261-266.

Cooney, G.J., Denyer, G.S., Jenkins, A.B., Storlien, L.H., Kraegen, E.W. and Caterson, I.D. (1993). In vivo insulin sensitivity of the pyruvate dehydrogenase complex in tissues of the rat. Am. J. Physiol. 265 (1 Pt 1): E102-E107.

Cushman, S.W. and Wardzala, L.J. (1980). Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell. Apparent translocation of intracellular transport systems to the plasma membrane. J. Biol. Chem. 255: 4758-4762.

D'Elia, J.A., Weinrauch, L.A., Healy, R.W., Libertino, R.W., Bradley, R.F., Leland, O.S. (1979). Myocardial dysfunction without coronary artery disease in diabetic rental failure. Am. J. Cardiol. 43: 193-199.

Dai, S., Thompson, K.H. and McNeill, J.H. (1994a). One-year treatment of streptozotocin-induced diabetic rats with vanadyl sulphate. Pharmacol. Toxicol. 74: 101-109.

Dai, S. and McNeill, J.H. (1994b). One-year treatment of non-diabetic and streptozotocin-diabetic rats with vanadyl sulphate did not alter blood pressure or haematological indices. Pharmacol. Toxicol. 74: 110-115.

Dai, S., Thompson, K.H., Vera, E. and McNeill, J.H. (1994c). Toxicity studies on one-year treatment of non-diabetic and streptozotocin-diabetic rats with vanadyl sulphate. Pharmacol. Toxicol. 75: 265-273.

DeFronzo, R.A., Jacot, E., Jequier, E., Maeder, E., Wahren, J. and Felber J.P. (1981). The effect of insulin on the disposal of intravenous glucose: results from indirect calorimetry and hepatic and femoral venous catheterization. Diabetes 30: 1000-1007.

Denton, R.M. and Randle, P.J. (1967). Concentrations of glycerides and phospholipids in rat heart and gastrocnemius muscles. Biochem. J. 104: 416-422.

Dillmann, W.H. (1980). Diabetes mellitus induces changes in cardiac myosin in the rat. Diabetes. 29: 579-582.

Dillmann, W.H. (1982). Influence of thyroid hormone administration of myosin ATPase activity and myosin isoenzyme distribution in the heart of diabetic rats. Metabolism 31: 199-204.

Dillmann, W.H., Boheler, K.R., Popovich, B.K. (1988). Diabetes decreases creatinine kinase (CK) enzyme activity, CK-M and CK-B mRNA levels in the rat heart. Diabetes 37 (Suppl. 1): 46A.

Domingo, J.L., Llobet, J.M. Gomez, M., Corbella, J. and Keen, C.L. (1990). Metal ions in biology and medicine Eds. Ph. Collery, LA. Poirier, M. Manfaint, J.C. Etienne. John Libbey Eurotex, Paris 312-314.

Domingo, J.L., Gomez, M., Llobet, J.M., Corbella, J. and Keen, C.L. (1991a). Improvement of glucose homeostasis by oral vanadyl or vanadate treatment in diabetic rats is accompanied by negative side effects. Pharmacol. Toxicol. 68: 249-253.

Domingo, J.L., Ortega, A., Llobet, J.M. and Keen, C.L. (1991b). No improvement of glucose homeostasis in diabetic rats by vanadate treatment when given by gavage. Trace Elem. in Med. 8: 181-186.

Domingo, J.L., Gomez, M., Llobet, J.M., Corbella, J and Keen, C.L. (1991c). Oral vanadium administration to streptozotocin-diabetic rats has marked negative side-effects which are independent of the form of vanadium used. Toxicol. 66: 279-287.

Domingo, J.L., Sanchez, D.J., Gomez, M., Llobet, J.M. and Corbella, J. (1992). Administration of vanadyl sulphate by gavage does not normalize blood glucose levels in streptozotocin-induced diabetic rats. Res. Comm. Chem. Pathol. Pharmacol. 75: 369-372.

Dubyak, G.R. and Kleinzeller, A. (1980). The insulin-mimetic effects of vanadate in isolated rats adipocytes. Dissociation from effects of vanadate as a (Na^+-K^+)-ATPase inhibitor. J. Biol. Chem. 255 (11): 5306-5312.

Duckworth, W.C., Solomon, S.S., Liepnieks, J., Hamel, F.G., Hand, S. and Peavy, D.E. (1988). Insulin-like effects of vanadate in isolated rat adipocytes. Endocrinology 122: 2285-2289.

Eckel, J. and Reinauer, H. (1990). Insulin action on glucose transport in isolated cardiac myocytes: signaling pathways and diabetes induced alterations. Biochem. Soc. Trans. 18: 1135-1137.

Eckel, J., Asskamp, B., Reinauer, H. (1991). Induction of insulin resistance in primary cultured adult cardiac myocytes. Endocrinology 129: 345-352.

Fein, F.S., Kornstein, L.B., Strobeck, J.E., Capasso, J.M. and Sonnenblick, E.H. (1980). Altered myocardial mechanics in diabetic rats. Circ. Res. 47: 922-933.

Fein, F.S. and Sonnenblick, E.H. (1985). Diabetic cardiomyopathy. Prog. Cardiovasc. Res. 27: 255-270.

Fischer, Y., Rose, H. and Kammermeier, H. (1991). Highly insulin-responsive isolated rat heart muscle cells yield by a modified isolation method. Life Sci. 49: 1679-1688.

Galderisi, M., Anderson, K.M., Wilson, P.W.F. and Levy, D. (1991). Echocardiographic evidence for the existence of a distinct diabetic cardiomyopathy (The Framingham Heart Study). Am. J. Cardiol. 68: 85-89.

Ganguly, P.K., Pierce, G.N., Dhalla, K.S. and Dhalla, N.S. (1983). Defective sarcoplasmic reticular calcium transport in diabetic cardiomyopathy. Am. J. Physiol. 244: E528-535.

Garland, P.B., Newsholme, E.A. and Randle, P.J. (1962). Effect of fatty acids, ketone bodies, diabetes and starvation on pyruvate metabolism in rat heart and diaphragm muscle. Nature Lond. 195: 381-383.

Garvey, W.T., Huecksteadt, T.P. and Birnbaum, M.J. (1989). Pretranslational suppression of an insulin-responsive glucose transporter in rats with diabetes mellitus. Science 245: 60-63.

Gil, J., Miralpeix, M., Carreras, J. and Bartrons, R. (1988). Insulin-like effects of vanadate on glucokinase activity and fructose 2,6-bisphosphate levels in the liver of diabetic rats. J. Biol. Chem. 263: 1868-1871.

Glatz, J.F.C., van Breda, E., Keizer, H.A., de Jong, Y.F., Lakey, J.R.F., Rajotte, R.V., Thompson, A., va der Vusse, G.J. and Lopaschuk, Gd. (1994). Rat

heart fatty acid-binding protein content is increased in experimental diabetes. Biochem. Biophys. Res. Commun. 199: 639-646.

Goldfine, A. B., Folli., F., Patti, M.E., Simonson, D.C. and Kahn, C.R. (1994). Effects of sodium vanadate on *in vivo* and *in vitro* insulin action in diabetes. Can. J. Physiol. Pharmacol. 72 (3): L16.

Hamby, R.L., Zoneraich, S. and Sherman, L. (1974). Diabetic cardiomyopathy. J. Am. Med. Assoc. 229: 1749-1754.

Herr, R.R., Eble, T.E., Bergy, M.E. and Jahnke, H.K. (1960). Isolation and characterization of Streptozotocin. Antibiot Ann 1959-1960, New York Medical Encyclopedia, Inc., 236.

Heyliger, C. E., Tahiliani, A.G. and McNeill, J.H. (1985). Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats. Science (Washington, D.C.) 227: 1474-1477.

Heyliger, C.E., Prakash, A. and McNeill, J.H. (1987). Alterations in cardiac sarcolemmal Ca^{2+} pump activity during diabetes mellitus. Am. J. Physiol. 252: H540-H544.

Hutson, N.J., Kerbey, A.L., Randle, P.J. and Sugden, P.H. (1978). Conversion of inactive (phosphorylated) pyruvate dehydrogenase complex into active complex by the phosphate reaction in heart mitochondria is inhibited by alloxan-diabetes or starvation in the rat. Biochem. J. 173 (2): 669-680.

Ingebretsen, C. P., Moreau, P., Hawelu-Johnson, C. and Ingebretsen, W. R. (1980). Performance of diabetic rat hearts: effects of anoxia and increased work. Am. J. Physiol. 239 (Heart Circ. Physiol. 8): H614-620.

Jenkins, R.L., McDaniel, H.G., Digerness, S., Parrish, S.W., Ong, R.L. (1988). Adenine nucleotide mechanism in heart of diabetic rats: comparison to diaphragm, liver, and kidney. Diabetes 37: 629-636.

Junod, A., Lambert, A.E., Orci, L., Pictet, R., Gonet, A.E. and Renold, A.E. (1967). Studies of the diabetogenic action of streptozotocin. Proc. Soc. Exp. Bio. Med. 126: 201.

Kadota, S., Fantus, G., Deragon, G., Guyda, H.J., Hersh, B. and Posner, B.I. (1987). Peroxides of vanadium: a novel and potent insulin-mimetic agent that activates the insulin receptor kinase. Biochem. Biophys. Res. Commun. 147: 259-266.

Kannel, W.B., Hjortland, M. and Castelli, W.P. (1974). Role of diabetes in congestive heart failure: the Framingham study. Am. J. Cardiol. 34 (1): 29-34.

Karnieli, E., Zarnowski, M.J., Hissin, P.J., Simpson, I.A., Salans, L.B. and Cushman, S.W. (1981). Insulin-stimulated translocation of glucose transport systems in the isolated rat adipose cell. J. Biol. Chem. 256: 4772-4777.

Karnieli, E., Armoni, M., Cohen, P., Kanter, Y. and Rafaeloff, R. (1987). Reversal of insulin resistance in diabetic rat adipocytes by insulin therapy: restoration of pool of glucose transporters and enhancement of glucose-transport activity. Diabetes 36: 925-931.

Kobayashi, M. and Olefsky, J.M. (1979). Effects of streptozotocin-induced diabetes on insulin binding, glucose transport and intracellular glucose metabolism in isolated rat adipocytes. Diabetes 28: 87-95

Kryski, A.Jr., Kenno, K.A. and Severson, D.L. (1985). Stimulation of lipolysis in rat heart myocytes by isoproterenol. Am. J. Physiol. 248: H 208-216.

Latipaa, P.M., Peuhkurinen, K.J., Hiltunen, J.K. and Hassinen, I.E. (1985). Regulation of pyruvate dehydrogenase during infusion of fatty acids of varying chain lengths in the perfused rat heart. J. Mol. Cell. Cardiol. 17: 1161-1171.

Ledet, B., Neubauer, B., Christensen, N.J., Lundback, K. (1979). Diabetic cardiopathy. Diabetologia 16: 207-209.

Llobet, J.M. and Domingo, J.L. (1984). Acute toxicity of vanadium compounds in rats and mice. Toxicol. Lett. 23: 227-231.

Lopaschuk, G.D., Tahiliani, A.G., Vadlamudi, R., Katz, S. and McNeill, J.H. (1982). The effect of alloxan and streptozotocin-induced diabetes on calcium transport in rat sarcoplasmic reticulum. The possible involvement of long chain acylcarnitines. Can J. Physiol. Pharmacol. 61: 439-448.

Lopaschuk, G.D. and Gamble, J. (1994). Acetyl-CoA carboxylase: an important regulator of fatty acid oxidation in the heart. Can. J. Physiol. Pharmacol. 72: 1101-1109.

Malhotra, A., Penpargkul, S., Fein, F.S., Sonnenblick, E.H. and Scheuer, J. (1981). The effect of streptozotocin-induced diabetes in rats on cardiac contractile proteins. Circ. Res. 49: 1243-1250.

McNeill, J.H., Yuen, V.G., Hoveyda, H.R. and Orvig, C. (1992). Bis(maltolato)oxovanadium(IV) is a potent insulin mimic. J. Med. Chem. 35: 1489-1491.

Meerson, F.Z. (1969). The myocardium in hypertension, hypertrophy and heart failure. Circ. Res. 25 (suppl II): 1.

Meyerovitch, J., Farfel, Z., Sack, J. and Shechter, Y. (1987). Oral administration of vanadate normalizes glucose levels in streptozotocin-treated rats. J. Biol. Chem. 262 (14): 6658-6662.

Mongold, J.J., Cros, G.H., Vian, L., *et al.* (1990) Toxicological aspects of vanadyl sulphate treatment of diabetic rats. Pharmacol. Toxicol. 67: 192-198.

Murer, E., Boden, G., Gyda, M. and Deluca, F. (1992). Effects of oleate and insulin on glucose uptake, oxidation, and glucose transporter proteins in rat adipocytes. Diabetes 41: 1063-1068.

Murthy, V.K. and Shipp, J.C. (1977). Accumulation of myocardial triacylglycerols in ketotic diabetes. Diabetes 26: 222-229.

Neely, J.R. and Morgan, H.E. (1974). Relationship between carbohydrate metabolism and energy balance of heart muscle. Annu. Rev. Physiol. 36: 413-459.

Nicholl, T.A., Lopaschuk, G.D., McNeill, J.H. (1991). Effects of free fatty acids and dichloroacetate on isolated working diabetic rat heart. Am. J. Physiol. 261: 1053-1059.

Nielsen, F.H. and Uthus, E.O. (1990). The essentially and metabolism of vanadium. In Vanadium in Biological systems, physiology and biochemistry. Edited by N.D. Chasteen. Kluwer Academic Publisher, London. 51-62.

Nuutila, P., Koivisto, V.A., Knuuti, J., Ruotsalainen, M., Teras, M., Haaparanta, M., Bergman, J., Solin, O., Voipio-Pulkki, L.M., Wegelius, U. and Yki-Jarvinen, H. (1992). Glucose-free fatty acid cycle operates in human heart and skeletal muscle in vivo. J. Clin. Invest. 89: 1767-1774.

Opie, L.H. (1970). Effect of fatty acid on contractility and rhythm of the heart. Nature (Lond.) 227: 1055-1056.

Pederson, R.A., Ramanadham, S., Buchan, A.M.J. and McNeill, J.H. (1989). Long-term effects of vanadyl treatment on streptozotocin-induced diabetes in rats. Diabetes 38: 1390-1395.

Penpargkul, S., Schaible, T., Yiptinsoi, T. and Scheuer, J. (1980). The effect of diabetes on performance and metabolism of rat heart. Circ. Res. 47: 911-921.

Penpargkul, S., Fein, F., Sonnenblick, E.H. and Scheuer, J. (1981). Depressed cardiac sarcoplasmic reticular function from diabetic rats. J. Mol. Cell. Cardiol. 13: 303-309.

Pierce, G. N. and Dhalla, N.S. (1983). Sarcolemmal Na^+-K^+-ATPase activity in diabetic rat heart. J. Mol. Cell. Cardiol. 245: C241-C247.

Pierce, G.N., Kutryk, M.J.B. and Dhalla, N.S. (1983). Alterations in Ca^{2+}-binding by and composition of the cardiac sarcolemmal membrane in chronic diabetes. Proc. Natl. Acad. Sci. USA. 80: 5412-5416.

Posner, B.I., Faure, R., Burgess, J.W., Bevan, A.P., Lachance, D., Zhang-Sun, G., Fantus, I.G., Ng, J.B., Hall, D.A., Soo Lum, B. and Shaver, A. (1994). J. Biol. Chem. 269: 4596.

Ramanadham, S., Mongold, J.J., Brownsey, R.W., Cros, G.H. and McNeill, J.H. (1989). Oral vanadyl sulphate in treatment of diabetes in rats. Am. J. Physiol. 257: H904-H911

Ramanadham, S., Cros, G.H., Mongold, J.J., Serrano, J.J., and McNeill, J.H. (1990). Enhanced in vivo sensitivity of vanadyl-treated diabetic rats to insulin. Can. J. Physiol. Pharmacol. 68: 486-491.

Randle, P.J., Hales, C.N., Garland, P.B. and Newsholme, E.A. (1963). The glucose fatty acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1: 785-9.

Regan, T.J., Ettinger, P.O., Khan, M.I., Jesrani, M.U., Lyons, M.M., Oldewurtel, H.A. and Weber, M. (1974). Altered myocardial function and metabolism in chronic diabetic models without ischemia in dogs. Circ. Res. 35: 222-237.

Regan, T.J., Lyons, M.M., Ahmed, S.S. (1977). Evidence for cardiomyopathy in familial diabetes mellitus. J. Clin. Invest. 60: 885-889.

Rizza, R.A., Crass, M.F. and Shipp, J.C. (1971). Effect of insulin treatment in vivo on heart glycerides and glycogen of alloxan-diabetic rats. Metabolism 20: 539-543.

Rodrigues, B., Goyal, R.K. and McNeill, J.H. (1986). Effects of hydralazine treatment on streptozotocin-induced diabetic rats: prevention of

hyperlipidemia and improvement in cardiac function. J. Pharmacol. Exp. Ther. 237: 292-299.

Rodrigues, B., Xiang, H. and McNeill, J.H. (1988). Effects of L-carnitine treatment on lipid metabolism and cardiac performance in chronic diabetic rats. Diabetes 37: 1358-1364.

Rodrigues, B. and McNeill, J.H. (1992). The diabetic heart: metabolic causes for the development of cardiomyopathy. Cardiovasc. Res. 26: 913-922.

Rodrigues, B., Spooner, M. and Severson, D.L. (1992). Free fatty acids do not release lipoprotein lipase from isolated cardiac myocytes or perfused hearts. Am. J. Physiol. 262 (2 Pt1): E216-223.

Rodrigues, B. and Grassby, P.F., Battell, M.L., Lee, S.Y.N. and McNeill, J.H. (1994). Hypertriglyceridemia in experimental diabetes: relationship to cardiac dysfunction. Can. J. Physiol. Pharmacol. 72: 447-455.

Rodrigues, B., Cam, C.C. and McNeill, J.H. (1995). Myocardial Substrate metabolism: implications for diabetic cardiomyopathy. J. Mol. Cell. Cardiol. 27: 169-179.

Rosen, P., Adrian, M., Feuerstein, J. and Reinauer, H. (1984). Glycolysis and glucose oxidation in the rat heart under nonrecirculating perfusion conditions. Basic Res. Cardiol. 79: 307-312.

Rosen, P., Reinauer, P. (1984). Inhibition of carnitine palmitoyltransferase 1 by phenylalkyloxiranecarboxylic acid and its influence on lipolysis and glucose metabolism in isolated, perfused hearts of streptozotocin-diabetic rats. Metabolism 33: 177-184.

Rosen, P., Windeck, P., Zimmer, H.G., Frenzel, H., Burrig, K.F. and Reinauer, H. (1986). Myocardial performance and metabolism in nonketotic, diabetic rat hearts: myocardial function and metabolism *in vivo* and in the isolated

perfused heart under the influence of insulin and octanoate. Bas. Res. Cardiol. 81: 620-635.

Rossetti, L., Cohen, N., Halberstam, M., Shimovich, P., Hu, M. and Shamoon, H. (1994). In vivo metabolic effects of vanadium on skeletal muscle and hepatic glucose metabolism. Can. J. Physiol. Pharmacol. 72 (3), L17.

Rubler, S., Dlugash, J., Yuceoglu, Y.Z., Kumral. T., Branwood, A.W. and Grishman, A. (1972). New type of cardiomyopathy associated with diabetic glomerulosclerosis. Am. J. Cardiol. 30: 595-602.

Sabbioni, E. and Marafante, E. (1978). Metabolic patterns of vanadium in the rat. Bioinorg. Chem. 9: 389-407.

Saddik, M. and Lopaschuk, G.D. (1991). Myocardial triglyceride turnover and contribution to energy substrate utilization in isolated working rat hearts. J. Biol. Chem. 266: 8162-8170.

Saddik, M. and Lopaschuk, G.D. (1994). Triacylglycerol turnover in isolated working hearts of acutely diabetic rats. Can. J. Physiol. Pharmacol. 72: 1110-1119.

Schulz, H. (1991). Beta-oxidation of fatty acids. Biochim. Biophys. Acta. 1081: 109-120.

Schwartz, K., Lecarpentier, Y., Martin, J.L. et al. (1981). Myocardial isoenzymic distribution correlated with speed of myocardial contraction. J. Mol. Cell. Cardiol. 13: 1071-1075.

Sekar, A., Kanthasamy, S. William, Subramanian, S. and Gouindasamy, S. (1990). Insulin-like effect of vanadium ion on streptozotocin-induced diabetic rats. J. Endocrinol. 126: 451-459.

Shechter, Y. and Karlish, S.J.D. (1980). Insulin-like stimulation of glucose oxidation in rat adipocytes by vanadyl (IV) ions. Nature 284: 556-558.

Shechter, Y. and Ron, A. (1986). Effects of depletion of phosphate and bicarbonate ions on insulin action in rat adipocytes. J. Biol. Chem. 261: 14945-14950.

Shipp, J.C., Opie, L.H. and Challoner, D. (1961). Fatty acid and glucose metabolism in the perfused heart. Nature 189: 1018-1019.

Shisheva, A. and Shechter, Y. (1992). A cytosolic protein tyrosine kinase in rat adipocytes. FEBS Letters 300: 93-96.

Shisheva, A. and Shechter, Y. (1993). Role of cytosolic tyrosine kinase in mediating insulin-like actions of vanadate in rat adipocytes. J. Biol. Chem. 268: 6463-6469.

Stacpoole, P.W. and Greene, Y.J. (1992). Dichloroacetate. Diabetes Care 15: 785-791.

Strout, H. V., Vicario, P.P., Biswas, C., *et al.* (1990). Vanadate treatment of streptozotocin diabetic rats restores expression of the insulin responsive glucose transporter in skeletal muscle. Endocrinology 126: 2728-2732.

Suzuki, K. and Kono, T. (1980). Evidence that insulin causes translocation of glucose transport activity to the plasma membrane from intracellular storage site. Pro. Natl. Acad. Sci. USA., 77: 2542-2545.

Swarup, G., Cohen, S. and Garber, D.L. (1982). Inhibition of membrane phosphotyrosyl-protein phosphatase activity by vanadate. Biochem. Biophys. Res. Comm. 107: 1104-1109.

Tahiliani, A.G., Vadlamudi, R.V.S.V. and McNeill, J.H. (1983). Prevention and reversal of altered myocardial function in diabetic rats by insulin treatment. Can. J. Physiol. Pharmacol. 61 (5): 516-523.

Tahiliani, A.G. and McNeill, J.H. (1985). Lack of effect of thyroid hormone on diabetic rat heart function and biochemistry. Can. J. Physiol. Pharmacol. 62: 902-911.

Tamura, S., Brown, T.A., Whipple, J.H., *et al.* (1984). A novel mechanism for the insulin-like effect of vanadate on glycogen synthase in rat adipocytes. J. Biol. Chem. 259: 6650-6658.

The University Group Diabetes Program (1975). A study of the effects of hypoglycemic agents on vascular complications in patients with adult-onset diabetes. V. Evaluation of pheniformin therapy. Diabetes 24 (Suppl 1): 65-184.

Underwood, E.J. (1977). Vanadium in trace elements in human and animal nutrition. Academic Press, New York. 388-397.

Valera, A., Rodriguez-Gil, J.E., and Bosch, F. (1993). Vanadate treatment restores the expression of genes for key enzymes in the glucose and ketone bodies metabolism in the liver of diabetic rats. J. Clin. Invest. 92: 4-11.

van der Vusse, G.J., Glatz, J.F.C., Stam, H.C.G. and Reneman, R.S. (1992). Fatty acid homeostasis in the normoxic and ischemic heart. Physiol. Rev. 72: 881-940.

Wall, S.R. and Lopaschuk, G.D. (1989). Glucose oxidation rates in fatty acid-perfused isolated working hearts from diabetic rats. Biochim. Biophys. Acta. 1006: 97-103.

Wieland, O., Siess, E., Schulze-Wethmar, F.H., Funcke, H.G. von., Winton, B. (1971). Active and inactive forms of pyruvate dehydrogenase in rat heart and dehydrogenase interconversion. Arch. Biochem. Biophys. 143 (2): 593-601.

Yuen, V.G., Orvig, C. and McNeill, J.H. (1993a). Glucose-lowering effects of a new organic vanadium complex, bis(maltolato)oxovanadium (IV). Can. J. Physiol. Pharmacol. 71: 263-269.

Yuen, V.G., Orvig, C. and McNeill, J.H. (1993b). Glucose-lowering effects of a new organic vanadium complex, bis(maltolato)oxovanadium (IV). Can. J. Physiol. Pharmacol. 71: 263-269.

Yuen, V.G., Orvig, C. and McNeill, J.H. (1995). Comparison of the glucose lowering properties of vanadyl sulphate and bis(maltolato)oxovanadium (IV) following acute and chronic administration. Can. J. Physiol. Pharmacol. 73: 55-64.