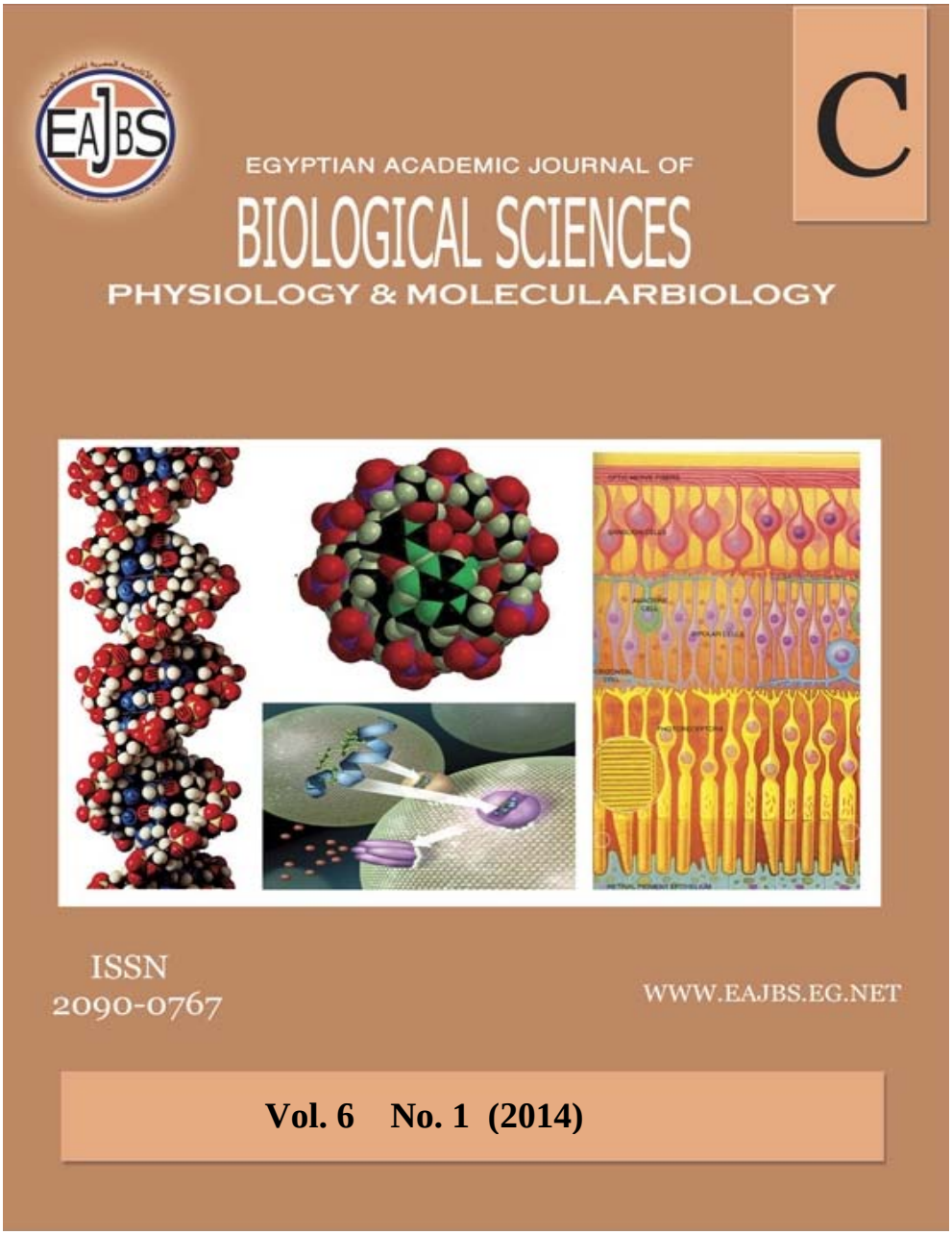

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The impact of vanadium on endothelial dysfunction in type 2 diabetic rats: Histological insight

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ABSTRACT

Introduction: The aim of this research is to investigate the effect of vanadium and or insulin on endothelial dysfunction in type 2 diabetic rats (T2DM).

Material and methods:60 white albino rats were included in this research and randomly divided into 6 groups(n=10) as follows: Control group (Control): rats injected once intraperitoneally (i.p.) with citrate buffer (0.1 M, pH 4.5), vanadium treated group (Vanadium): rats received vanadyl sulfate of 0.64 mmol/kg weight freshly dissolved in 1 ml of distilled water daily through a nesophageal tube, diabetic type 2group(D type 2) : rats received high fat diet for 15 days followed by streptozotocin (25 mg/kg body weight), diabetic type 2 and insulin group (D type 2 +I): D type 2 rats received mixtard insulin subcutaneously in a dose of 0.75 IU/100 gm weight in 0.75 ml volume once daily, diabetic type 2 and vanadium group (D type 2+V): type 2 diabetic ratsreceived the same dose of vanadium as in vanadium group after 48 h of induction of type 2 diabetes, diabetic type 2 + insulin + vanadium group (D type 2 +V+I): diabetic type 2 rats received both vanadium and insulin with the same doses as in previous groups.

Results: Diabetic group showed hyperglycemia, increased cholesterol(C), triglycerides (TG), LDL-C and decrease HDL-C. Administration of both insulin and vanadium caused significant decrease of glucose, TG, C, LDL-C and increased HDL-C back to control level. Histological analysis of aortae documented our results.

Conclusion: Administration of vanadium enhances the effect of insulin and restore endothelial damage in T2DM.

INTRODUCTION

Diabetes mellitus (DM) is a serious metabolic disease that affects over 171 million individuals worldwide (World Health Organization, National Institutes of Health).

T2DM, known as adult-onset or non-insulin-dependent diabetes mellitus, is characterized by insulin resistance in peripheral tissues, and may also be accompanied by a reduction in insulin secretion by the beta cells of the pancreas. T2DM accounts for approximately 85% of all cases of DM worldwide. Genetics is thought to play a much greater role in T2DM compared to T1DM. Unlike T1DM, autoimmunity does not play a role in the initial development of T2DM, while lifestyle factors such as obesity, smoking, alcohol consumption, and a sedentary lifestyle correlate significantly with the development of T2DM(Mozaffarian *et al*. 2009).In addition to lifestyle modifications and diet restriction to promote weight loss, T2DM is most often treated with multiple oral medications, and less frequently exogenous insulin. There are multiple classes of approved drugs, each with different mechanisms of action, however ultimately the goal of these therapies is to normalize plasma blood glucose levels and reduce cardiovascular risk factors (Ripsin, Kang and Urban 2009).

Type 2 diabetes is a strong risk factor for cardiovascular disease (CVD), with some studies suggesting that it confers an equivalent risk to having had a myocardial infarction (Haffner *et al*. 1998).Multifactorial interventions, such as the Steno-2 study, have been effective in reducing the risk of non-fatal and fatal CVD among diabetic patients through therapy targeting hyperglycaemia, hypertension and hypercholesterolaemia(Gaede *et al*. 2008).

Vanadium, as a trace element with insulinomimic effect, may act synergistically with insulin to protect against the development of DM. Vanadium is a transitional element that is widely distributed in nature and its oral administration has been reported to improve DM in humans(Gaede *et al.* 2008) The glucose-lowering effect of vanadium is due to its insulin mimetic properties. It is not the only mechanism through which vanadium improves DM, but a variety of different mechanisms have been suggested (Karmaker *et al*. 2007).Vanadium may mediate its action through changing of insulin sensitivity in the liver, kidney, and other tissues (Fantus and Tsiani 1998).Vanadium not only has insulinomimic action, but also enhances insulin activities and its cellular bioavailability (Karmaker *et al*. 2007.

The aim of the present work is to investigate the impact of vanadium alone or as an adjuvant treatment with insulin on endothelial dysfunction in T2DM. .

MATERIALS AND METHODS

Animals

The study was approved by the ethical committee of College of Medicine, King Khalid University, Abha, Saudi Arabia.

Induction of diabetes mellitus:

The Induction of T2DM: Rats received high-fat diet (HFD) (58% calories as fat) for a period of 2 weeks. After 2 weeks rats were injected intraperitoneally (ip) with low dose of streptozotocin (STZ) at 35 mg /kg body weight (Srinivasan *et al*. 2005) . DM will be verified by measuring blood glucose through tail–neck blood sampling. Rats with non-fasting blood glucose level of ≥20 mmol/L will be considered to be diabetic (Kedziora-Kornatowska *et al*. 1998). The study period of the experiment will be finished after 30 days. The failure rate for development of diabetes was expected to be 15% and the death rate was up to 5%, The failed and died rats was excluded from the experimental study group from the start.

Animals groups

This study followed a randomized controlled animal experiment design. A total of 60 male Sprague-Dawley rats were included in this study. All rats obtained from the animal house of King Khalid University and weighed between 150 and 200 gm. Animals fed on a standard rat chow and water, *ad libitum* and housed in the animal house of king Khalid University with a 12:12-hrs light/dark cycle. The rats were

randomly allocated to the study and divided into 6 groups (n = 10 each) as follows:

Control group (Control): rats will injected intraperitoneally (i.p.) once with citrate buffer (0.1 M, pH 4.5).

Vanadium treated group (Vanadium): rats injected i.p. with buffer as C group and received vanadyl sulfate of 0.64 mmol/kg weight freshly dissolved in 1 ml of distilled water daily through a nesophageal tube (Yuen, Orvig and McNeill 1995).

Diabetic type 2 group (D type 2): rats received high fat diet for 15 days followed by streptozotocin (25 mg/kg body weight), (Srinivasan *et al*. 2005).

1- Diabetic type 2 and insulin group (D type 2 +I): rats were made type 2 diabetic as in D type 2 group and received mixtard insulin subcutaneously in a dose of 0.75 IU/100 gm weight in 0.75 ml volume once daily (Unlucerci *et al*. 2002), after 48 h of induction of diabetes.
2- Diabetic type 2 and vanadium group (D type 2+V): type 2 diabetic rats received the same dose of vanadium as in vanadium group after 48 h of induction of type 2 diabetes.
3- Diabetic type 2, insulin and vanadium group (D type 2 +V+I): diabetic type 2 rats received both vanadium and insulin with the same doses as in previous groups.

Parameters

A. Biochemical and Metabolic Parameters:

At the end of the 4th weeks of the experiment, 5 ml retro-orbital blood samples will be obtained under anesthesia using 40 $mgKg^{-1}$ sodium thiopentone, ip, after an overnight fast**.** The collected blood allowed to clot for 20 minutes then centrifuged at 14000 rpm for 10 minutes for serum separation then stored at–80°C, for subsequent measurements of biochemical parameters (Fasting glucose, triacylglycerol (TG), total cholesterol., high density lipoprotein (HDL)-cholesterol and low density lipoprotein (LDL)-cholesterol.All measurement followed the manufacture instructionfor each specific kit parameter.

Histological Analysis:

In all animal groups, after withdrawal of the blood samples, the abdominal walls were opened and the abdominal aortae obtained. The aortae tissues were fixed in formalin and kept for histological diagnosis.

Statistical analysis

Data were expressed as frequency, percentage and mean ± SD. Testing significance will be performed using the one-way analysis of variance (ANOVA). P-values < 0.05 will considered statistically significant.

RESULTS

Our results showed hyperglycaemiain diabetic type 2 rats when compared with the control. Administration of vanadium alone or combined with insulin brings glucose back to control level [Fig.1 a]. Diabetic rats also showed increased level of cholesterol in comparison to control group. Administration of vanadium alone or combined with insulin showed decreased level of cholesterol in comparison to control group [Fig.1 b].

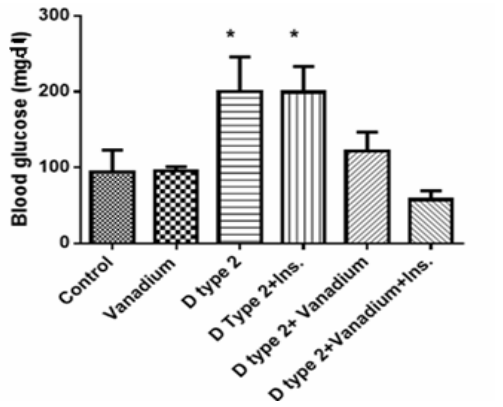

Fig. 1 a: Levels of blood glucose in different groups studies

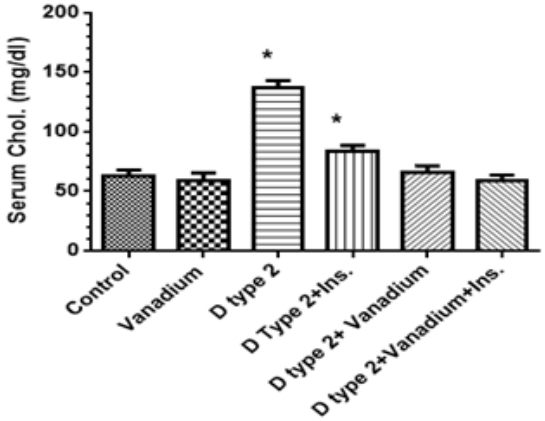

Fig.1 b: Levels of serum cholesterolin different groups

Figure 2 a showed decreased level of HDL-C in diabetic group. Administration of insulin or vanadium alone does affect this level, while administration of both vanadium and insulin caused significant increase in HDL-C. Figure 2 b also showed increased level of LDL-C in diabetic group in comparison to control. Administration of vanadium alone or combined with insulin decreased this level.

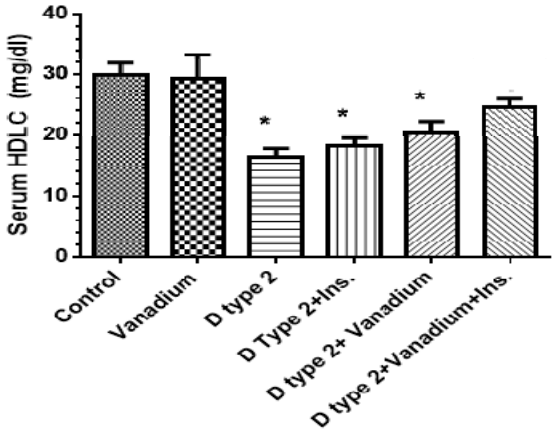

Fig. 2 a: Levels of serum HDL-C in different groups studied

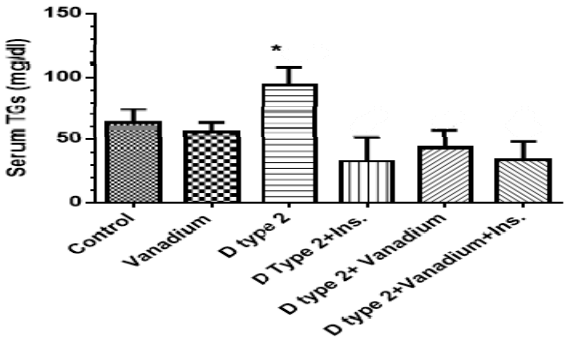

Fig. 2 b: Levels of serum LDL-C in different groups studied

Figure 3 showed increased TG in diabetic group. Administration of vanadium, insulin or combined vanadium and insulin caused significant decrease of TG back to control level.

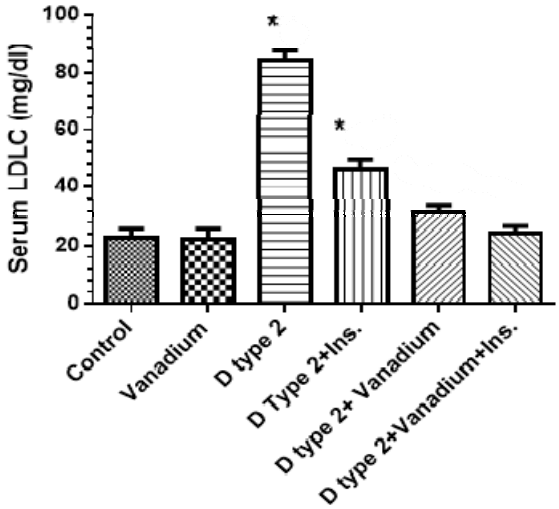

Fig. 3: Levels of serum TGs in different groups studies

Histological examination of aortae section of different groups, [Fig. 4a, B,C,D,E], showed degeneration of endothelium of type 2 diabetic rats Administration of insulin alone showed slight improvement , while administration of combined vanadium and insulin showed more improvement with intact smooth muscle cells , endothelial layer and lamina.

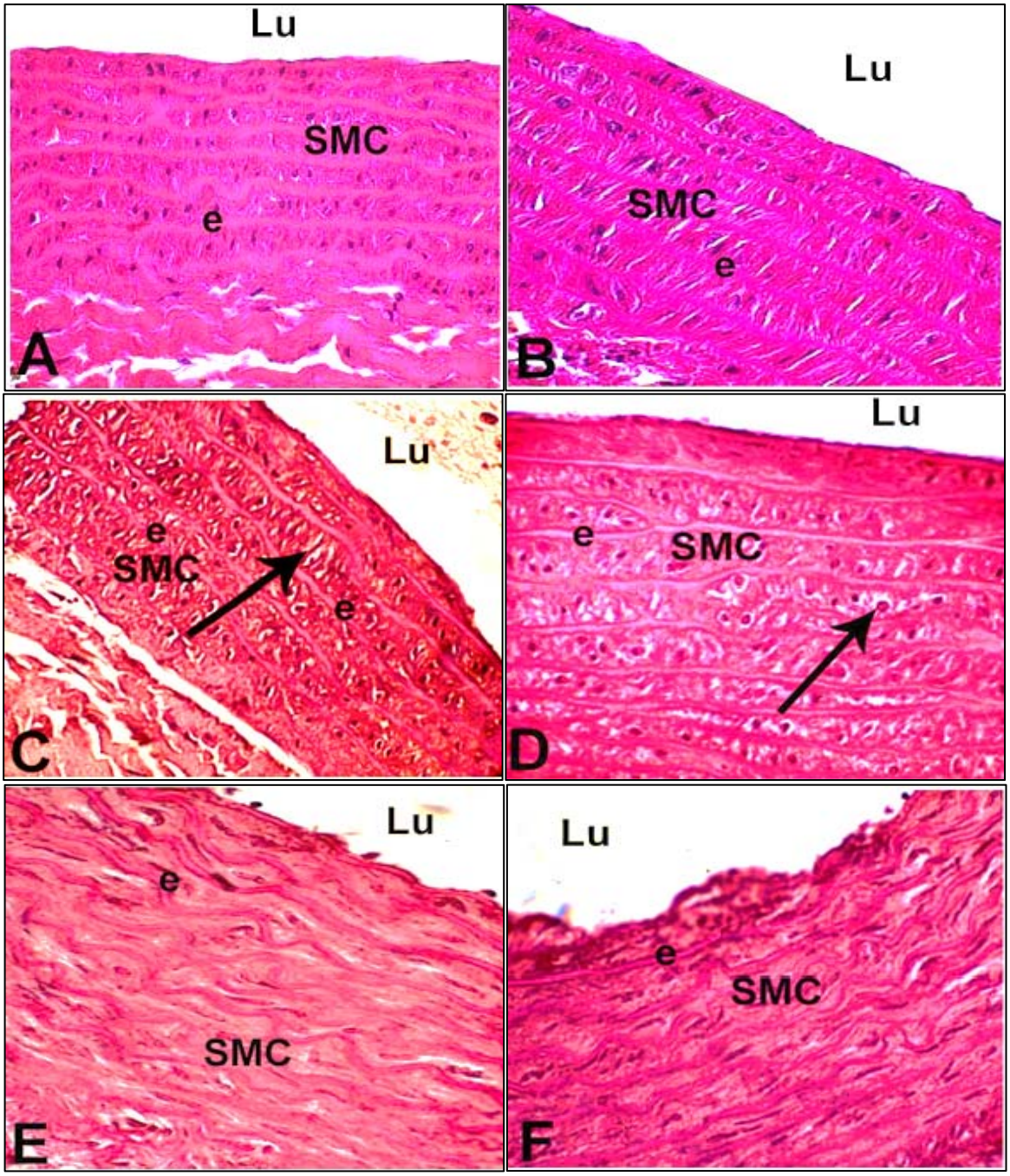

Fig. 4: Light micrographs of aortal cross section of normal control rats, stained by H&E.

Fig. 4 A: Cross section of control rats showing endothelial layer, elastic lamina (e) and smooth cells (SMC). Lu, lumen. (X400).

Fig. 4 B: Cross section of aorta of vanadium-treated rats showing endothelial layer, elastic lamina (e) and smooth cells (SMC). Lu, lumen. (X400).

Fig. 4 C: Cross section of aorta T2DM rats showing endothelium and degenerated smooth muscle cells (SMC) with vacuoles (arrow). Note elastic lamina (e) and lumen (Lu). (X400).

Fig. 4 D: Cross section of aorta of T2DM rats treated with insulin showing few degeneration of some smooth muscle cells (SMC) with vacuoles (arrow). Note elastic lamina (e) and lumen (Lu). (X400).

Fig. 4 E: Cross section ofT2DM rats treated with vanadium showing intact smooth muscle cells (SMC), endothelial layer (arrow) and elastic lamina. Lu, lumen. (X400).

Fig. 4 F: Cross section of aorta of T2DM rats treated with vanadium and insulin showing intact smooth muscle cells (SMC), endothelial layer (arrow) and elastic lamina (e). Lu, lumen. (X400)

DISCUSSION

T2DM is a chronic metabolic disorder characterized mainly by hyperglycemiawhich results from either a decrease in insulin secretion or increased insulin resistance in the target tissues (Tian *et al*. 2006). Diabetes mellitus is the major cause of many serious complications such as cardiovascular disorders, diabetic foot (Boulton *et al*. 2005), peripheral neuropathy, and nephropathy (Yin *et al*. 2004).

In the present study type 2 diabetes was induced in rats by administration of high fat diet for 2 weeks then rats injected with a single i.p. injection of STZ 35 mg/kg in freshly prepared citrate buffer (0.1 M, pH 4.5) (Reed *et al*. 2000, Srinivasan *et al*. 2005).

Despite the availability of insulin and a host of oral hypoglycaemic drugs, diabetes mellitus still remains a major health concern for humans. According to the WHO, it is estimated that approximately 150 million people worldwide have diabetes mellitus at present, and this number might double by 2025 (Groop 1992). Therefore, new therapeutic approaches are needed to treat diabetic complications more efficiently. In this regard, studies performed in the last two decades, which have been reviewed in detail. The authors demonstrated that compounds of the trace element vanadium exert various insulino-mimetic and anti-diabetic effects in vitro and in vivo (Cam, Brownsey and McNeill 2000, Srivastava 2000).

Vanadium, a group V trace element that belongs to the first transition series of elements, is universally distributed and represents the 21st most abundant element in the earth's crust (Nechay 1984). The element can exist in four valiancy states, 2, 3, 4 and 5, and, thus, its chemistry is complex. Vanadium occurs as vanadyl (VO2+) below pH 3.5, and in basic solutions, its predominant form is orthovanadate, which is chemically similar to the phosphates. Metavanadate, the predominant species in body fluids (e.g. plasma), enters cells by an anion transport system and is reduced by glutathione to the VO2+ form (Nechay 1984). Exogenously administered vanadylsulphate (VS) and ammonium vanadate have been found to bind serum transferrin tightly, indicating that this protein may serve as a vanadium transporter(Chasteen *et al*. 1986).

Several inorganic vanadium compounds, similar to insulin were found to stimulate glucose transport and oxidation in adipocytes, increased glycogen synthesis in the rat diaphragm and hepatocytes, and inhibited gluconeogenesis in liver cells(Tolman *et al*. 1979). Since then, numerous studies have revealed various insulino-mimetic effects of vanadium compounds in vitro and in vivo, including the stimulation of glucose transport and glucose oxidation (Green 1986), glycogen synthesis(Strout *et al*. 1989), lipogenesis as well as the inhibition of lipolysis (Shisheva and Shechter 1992) and gluconeogenesis (Tolman *et al*. 1979).

Among the in vivo actions of vanadium, the discovery that attracted the attention of diabetologists and endocrinologists was the seminal work of Heyliger *et al*. (Heyliger, Tahiliani and McNeill 1985) which showed that Na3VO4 normalized hyperglycaemia in an animal model of diabetes mellitus. Meyerovitch *et al*. (Meyerovitch *et al*. 1987)confirmed this observation, and it was suggested (Heyliger *et al*. 1985) that vanadium compounds could have potential in the treatment of diabetes mellitus. These findings were supported by several groups who extended them to animal models of T1DM and T2DM as well as humans.

The studies of Heyliger *et al*.(Heyliger *et al*. 1985) were confirmed and expanded by several other investigators(Srivastava 2000). In all these studies, hyperglycaemia was significantly reduced and, in many cases, virtually normalized by oral administration of vanadium compounds to diabetic rodents. The dose of vanadium salts required to exert a maximum glucose lowering action varied between studies, but a median concentration

of 0.5 mg/ ml in drinking water appeared to be sufficient.

The anti-diabetic potential of various vanadium compounds has also been examined in animal models of T2DM. Administration of NaOV, either in drinking water or in food in three well-characterized models of T2DM, genetically obese, fatty (fa/fa) Zucker rats, genetically diabetic C57 BL/KsJ-db/db (db/db) mice, and genetically diabetic ob/ob mice improved glucose homeostasis as well as oral glucose tolerance (Pugazhenthi, Angel and Khandelwal 1993).

Treatment of Psammomysobesus, a gerbil (nicknamed the 'sand rat') that represents a nutritionally induced model of diabetes and insulin resistance resulted in prolonged restoration of normoglycaemia andnormoinsulinemia. VS treatment in this model was associated with a normal glucose tolerance test and a decreased level of the hepatic gluconeogenic enzyme phosphoenol pyruvate carboxykinase (PEPCK). Interestingly, VS was ineffective when administered to sand rats that had lost their insulin secretory capacity, indicating a requirement of low-level insulin for vanadium to work in these animals. Thus, vanadium appears to be an insulin potentiator/ enhancer rather than a mimicker in improving insulin resistance in sand rats (Shafrir *et al*. 2001).

A potential insulin-enhancing role of vanadium has also been suggested from studies in which mildly diabetic rat models exhibited significant glucose-lowering effects with suboptimal doses of vanadium and insulin (Ramanadham *et al*. 1990). Nevertheless, vanadium possibly elicits its anti-diabetic response by a combination of insulino-mimetic and insulin-enhancing actions. Its insulin-sensitizing effect may be particularly important in the context of T2DM. It should be noted that thiazoldinediones, which function as insulin sensitizers, are already being used clinically to treat T2DM (Olefsky 2000).

The exact mechanism by which vanadium compounds improve hyperglycaemia and glucose homeostasis in diabetes remains unclear. Vanadium therapy in a Type 1 model of diabetes mellitus slightly but insignificantly increased plasma insulin (Heyliger *et al*. 1985, Meyerovitch *et al*. 1987) . A significant, up to 50% decrease of plasma insulin was observed in Type 2 models (Heyliger *et al*. 1985). This support our results which showed that administration of vanadium in T2DMdecreased blood glucose towards control level.

In addition to their action on glucose metabolism, vanadium compounds can modulate lipid metabolism both in vivo and in vitro. NaOV treatment of insulin-resistant, sucrose-fed diabetic rats and fa/faZucker rats significantly lowered plasma triglycerols (Khandelwal and Pugazhenthi 1995). Furthermore, VS decreased plasma cholesterol levels in humans (Cusi *et al*. 2001) without alteration of either plasma free fatty acid or triglyceride fractions(Curran, Azarnoff and Bolinger 1959) . Vanadate has also been shown to reduce total and free cholesterol levels in normal subjects(Curran *et al*. 1959), This is in support of our results which showed that administration of vanadium and or insulin decreased the levels of cholesterol, TG, LDL-Cholesterol , and increased the level of HDL-cholesterol.

Up to our best knowledge no one has published any original article that investigate the effect of vanadium on endothelial function in vascular system

Our results showed early increase of endothelial dysfunction markers and degeneration of endothelium in type 2 diabetic ratsone month after induction of diabetes, while administration of vanadium and or insulin restored smooth cells and elastic tissue back to control.

CONCLUSIONS

Compounds of the trace element vanadium exert obvious insulin- like effects. These include improvements in glucose homeostasis and insulin resistance in animals models ofT2DM.

As our results showed amelioration of disturbed carbohydrate and lipid profile in

diabetic rats. Meanwhile, we are the first to show improvement of markers endothelial dysfunction. We hope to extend our work to test the effect of administration of vanadium on endothelial dysfunction in human as this may help to prevent diabetic cardiovascular complications.

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