

Effect of Vanadyl Sulfate on Fructose-Induced Insulin Resistance Rat

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ABSTRACT

Insulin resistance syndrome, also referred to as the metabolic syndrome or syndrome X, refers to a constellation of common metabolic and cardiovascular disorders (e.g. obesity, type 2 diabetes mellitus, hypertension, and dyslipidemia), which are all cardiovascular risk factors. Insulin resistance can be induced by fructose-rich diet in rats. We investigated the effect of vanadyl sulfate (0.2 mg/ml in drinking water for 7 days) on glucose, triglyceride, and plasma insulin levels in male Wistar rats that were fed with fructose-rich diets. Control rats were fed with standard chow for 7 days. The animals were divided into three groups: fructose-fed rats, fructose fed-vanadyl sulfate treated rats, and control rats. Fasting plasma glucose levels of the three groups were comparable ($p>0.05$). Fasting plasma insulin increased in the fructose-fed rats (190 ± 6.3 pM vs. control rats 83.06 ± 3.3 pM, $p<0.001$), likewise, plasma triglyceride significantly increased in fructose-fed rats (394.0 ± 25.8 vs. control rats 98.63 ± 6.7 , $p<0.001$). Vanadyl sulfate treatment prevented the increase in plasma insulin levels in the fructose fed-vanadyl treated rats (78.9 ± 5.1 pM vs. fructose fed-groups, $p<0.001$). Also vanadyl sulfate treatment significantly decreased plasma triglyceride levels (116.43 ± 6.7 vs. fructose-fed rats, $p<0.001$). Furthermore, fructose-fed groups had higher fasting insulin resistance index (FIRI: $p<0.001$) than control rats. In contrast, vanadyl sulfate significantly decreased FIRI in the fructose fed- vanadyl treated groups ($p<0.001$) compared with fructose- fed animals. These results indicate that administration of low doses of vanadyl sulfate may be advantageous for preservation of the functional characteristics of pancreatic beta cells, probably by improving insulin action and thereby insulin resistance prevention. *Iran. Biomed. J. 7 (4): 179-182, 2003*

Keywords: Insulin resistance, Vanadyl sulfate, Diabetes mellitus, Fasting insulin resistance index (FIRI), Triglyceride

INTRODUCTION

Insulin resistance is a metabolic disorder that is increasing worldwide and plays a role in the pathophysiology of the most common human diseases including type 2 diabetes mellitus, hypertension, obesity, dyslipidemia and coronary heart disease [1]. The insulin resistant state is commonly associated with lipoprotein abnormalities that are risk factors for atherosclerosis, including hypertriglyceridemia, high levels of very low density lipoprotein (VLDL), low levels of high-density lipoprotein cholesterol [2], and small, dense LDL [3]. These metabolic abnormalities together with hypertension and type 2 diabetes mellitus may

cluster in the same individual, consisting a syndrome referred to as the metabolic syndrome X [4].

Type 2 diabetes mellitus or non-insulin-dependent diabetes mellitus (NIDDM) is characterized by progressive impairment of glucose homeostasis and sensitivity to insulin, particularly in skeletal muscle and liver [5]. Oral drug therapy aimed at controlling hyperglycemia in NIDDM often fails, and most patients require insulin treatment late in the course of the disease. This progressive deterioration in glucose metabolism is due, in part, to worsening insulin sensitivity, which may be ameliorated by the glucose-lowering effect of exogenous insulin therapy, but a number of side effects and

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complications accompany insulin therapy [6]. Therefore, agents, which could augment insulin sensitivity at the level of muscle and liver, may be useful in the treatment of insulin resistance in type 2 diabetes mellitus.

Recently the insolinomimetic properties of vanadium compounds have been documented *in vitro* and *in vivo* [7]. Vanadium is an ultra-trace element in mammals, and although several studies suggest that it is essential, at least in chicks and rats, its precise role in mammalian biology remains unknown [8]. Among its several biologic effects, vanadium has been shown to stimulate glucose uptake, glycogen synthesis, and glucose oxidation in adipose cells and in skeletal muscle [9]. Vanadium treatment also increased the basal activity of the insulin receptor tyrosine kinase [10]. Taking into account these observations, to ascertain whether treatment with vanadium could prevent insulin resistance-induced by fructose-enrich diet in Wistar rats, we undertaken to study the acute effects of small oral doses of vanadyl sulfate in insulin resistant animal models. Vanadyl sulfate was chosen because it is a form of vanadium that is less toxic than other forms such as vanadate [11, 12].

MATERIALS AND METHODS

Materials. Vanadyl sulfate ($\text{VO}(\text{SO}_4)_2$) was purchased from Merck Co. (Germany) and other chemicals were of analytical grade.

Assays. Plasma glucose and triglyceride determination were performed on an Auto Analyzer (Tecnichon Co. USA). Plasma insulin level was determined in duplicate using a double-antibody radioimmunoassay technique (Europe SA, Co. Belgium).

Animals and preparations. Eight-week-old male Wistar rats weighting 200-220 g were purchased from the Pasteur Institute of Iran (Tehran). Each group of rats was separately housed in a cage and had free access to water and food. Insulin resistance was induced in the rat by fructose-rich diet [13]. This diet contained 66% fructose, 20% casein, 12% soybean oil, 0.02% cholesterol, 0.7% necessary amino acids, and 1.28% vitamins and minerals [14]. Control rats were fed with standard laboratory chow (Pars Dam Co., Iran). At the beginning of the experiment, rats were divided into three groups: fructose fed rats ($n = 10$); fructose fed-vanadyl sulfate treated rats ($n = 10$), and control rats ($n = 8$). The control and fructose fed rats

receiving tap water, and fructose fed-vanadyl sulfate-treated rats receiving tap water supplemented with 0.2 mg/ml vanadyl sulfate that was prepared freshly every day. The treatment was carried out for 7 days. At the end of the treatment period (after an overnight fasting), blood samples were collected from retro-orbital plexus using microhematocrit tubes in liquid lithium heparin anti-coagulant and immediately centrifuged at $1,000 \times g$ for 15 min. Plasma was removed to measure glucose, triglyceride and insulin.

Insulin resistance calculation. Fasting insulin resistance index (FIRI) were calculated according to the formulas:

$$\text{FIRI} = \frac{\text{fasting insulin} \times \text{fasting glucose}}{25}$$

and

$$\text{FIRI} = \frac{\text{fasting insulin}}{\text{fasting glucose}}$$

Statistical data analysis. Results were expressed as mean \pm SEM. To confirm the normal distribution, the data were analyzed by one sample Kolmogorov-Smirnov's test and then by Levene's test and independent-samples student's *t*-test. Significance level was set at $p < 0.05$. All statistical analysis was performed using SPSS 9.0 for windows software system.

RESULTS

The effects of fructose feeding and vanadyl sulfate drinking on plasma glucose, triglyceride, and insulin level are shown in Table 1. The insulin resistance indices of different rat group are shown in Table 2. As shown in Table 1, fasting plasma glucose concentration of the three groups were comparable ($p > 0.05$) at the end of the treatment period. The treatment did not affect the plasma glucose levels in any group. In contrast, plasma level of triglyceride significantly increased in fructose-fed groups compared to control rats ($p < 0.001$). In addition, plasma triglyceride significantly decreased in fructose fed-vanadyl treated rats compared to fructose-fed group ($p < 0.001$). Fasting plasma insulin increased in the fructose-fed rats compared to control rats ($p < 0.001$). Vanadyl sulfate treatment prevented the increase in plasma insulin levels in the fructose fed-vanadyl

Table 1. Plasma glucose and insulin levels in the experimental rats (after 7 days). Values are mean \pm SEM. Comparisons were made by independent samples student's *t*-test.

Animal groups	Fasting plasma glucose (mg /dl)	Fasting plasma insulin (pM)	Fasting plasma ¹ TG (mg /dl)
Control (n = 8)	80.6 \pm 6.7	83.06 \pm 3.3	98.63 \pm 6.7
² F-F (n = 10)	*86.5 \pm 6.1	** 190.04 \pm 6.2	** 394.00 \pm 25.8
³ FF-VT (n = 10)	*82.3 \pm 6.2	***78.90 \pm 5.1	***116.43 \pm 10.0

* $P > 0.05$, ** $p < 0.001$, compared to control rats. *** $p < 0.001$, compared fructose-fed rats. ¹TG = Triglycerides, ²F-F = Fructose-fed, ³FF-VT = fructose fed-vanadyl treated.

treated rats, and therefore had significant effect on plasma insulin levels compared to fructose-fed groups ($p < 0.001$). In other word, in fructose fed-vanadyl treated rats, the plasma insulin levels reached those of the normoinulinemic control animals ($p > 0.05$).

On the other hand, fructose-fed groups had higher fasting insulin/fasting glucose ratio ($p < 0.001$) and higher FIRI ($p < 0.001$) than control rats (Table 2). In addition, vanadyl sulfate significantly decreased fasting insulin/fasting glucose ratio ($p < 0.001$) and FIRI in the fructose fed-vanadyl treated groups ($p < 0.001$) compared with fructose-fed animals. Vanadyl sulfate corrected insulin resistance indices, and therefore in fructose fed vanadyl-treated rats the fasting insulin/fasting glucose ratio and FIRI reached those of the control rats ($p > 0.05$).

Table 2. Insulin resistance indeices (fasting insulin/fasting glucose and FIRI) in experimental rats (after 7 days). Values are mean \pm SEM. Comparisons were made by independent samples student's *t*-test.

Animal groups	Fasting insulin/ fasting glucose	¹ FIRI
Control (n = 8)	1.03 \pm 0.07	267.8 \pm 28.5
² F-Fed (n = 10)	*2.20 \pm 0.30	*657.4 \pm 54.4
³ FF-VT (n = 10)	**0.96 \pm 0.08	** 259.7 \pm 27.8

* $p < 0.001$, compared to control rats. ** $p < 0.001$, compared to fructose-fed rats. ¹FIRI = (fasting insulin \times fasting glucose)/25; ²F-F = Fructose-fed; ³FF-VT = fructose fed-vanadyl treated.

DISCUSSION

The present study shows that fructose-induced significant hyperinsulinemia and hypertriglyceridemia in fasting rats (Table 1). The previous studies carried out in rats and other rodents (e.g., hamster) indicated the same results [13]. An adverse effect of fructose on insulin sensitivity of the rat is well-established. This phenomenon is believed to be related to the hypertriglyceridemic effect of fructose [15]. Also, results of present study indicate that fructose-induced hypertriglyceridemia is associated with significant hyperinsulinemia. Fructose feeding stimulates the hepatic production

of triglycerides, both by promoting the reesterification of circulating non-esterified fatty acids and by stimulating de novo fatty acid synthesis [16]. Increased delivery of triglycerides or non-esterified fatty acids to the muscle interferes with the utilization of glucose, through the principles of Randle cycle [17], impairing the insulin action. In contrast, three groups had comparable plasma glucose concentration, and the treatment did not affect plasma glucose levels in any group. In addition, our results showed that fructose-fed groups had higher fasting insulin/fasting glucose ratio ($p < 0.001$) and higher FIRI ($p < 0.001$) than control groups (Table 2).

Administration of Vanadyl sulfate (0.2 mg/ml in drinking water for 7 days) in fructose-fed rats significantly decreased plasma insulin, triglyceride, fasting insulin/fasting glucose ratio, and FIRI ($p < 0.001$, vs. fructose-fed rats) without any change in plasma glucose levels. Therefore, the available information strongly supports the close interrelationship between insulin resistance and hypertriglyceridemia [18]. These findings demonstrated that, enhancement of the sensitivity of target tissues to circulating insulin by vanadyl sulfate, might be related to lowering the plasma triglyceride.

Previous studies have shown that salts of the trace element vanadium, such as sodium orthovanadate and vanadyl sulfate, exhibit insulin-like effects, including stimulation of glycogen synthesis and improvement of glucose homeostasis in type 1 and type 2 animal models of diabetes mellitus [19]. However, the cellular mechanism by which these effects are mediated remains poorly characterized. Actually, high levels of blood circulating triglyceride interferes with insulin action due to its receptor [15], therefore our data demonstrate that the improvement of physiological insulin action and prevention of insulin resistance by vanadyl ion is due, in part, to increase insulin receptor binding. These findings do not agree with those reported by Pandey *et al.* [20], that showed vanadium salts ameliorated glucose homeostasis in diabetic rats and several isolated cell types, is independent of insulin receptor. Because of lake of any toxicity of

vanadium at low doses [11], therefore in future this trace element may come into use as a new drug for the management of diabetes patients.

In conclusion, our results shows that administration of low dose of vanadyl sulfate may be advantageous for preservation of the functional characteristic of pancreatic β cells, probably by improving insulin action and thereby prevent insulin resistance induction. However, further studies are needed to establish the safety and effectiveness of vanadyl ion in diabetes mellitus.

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REFERENCES

- Adeli, K., Taghibigluo, C.H., Van Iderstine, S.C. and Lewis, G.F. (2001) Mechanism of hepatic very low-density lipoprotein overproduction in insulin resistance. *Trends Cardiovasc. Med.* 11:170-176.
- Ruotolo, G. and Howard, B.V. (2002) Dislipidemia of the metabolic syndrome. *Curr. Cardiol. Rep.* 4 (6): 494-500.
- Friedlandre, Y., Kidorn, M., Caslake, M., Lamb, T., McConnell, M. and Bar-On, H. (2000) Low density lipoprotein particles size and risk factors of insulin resistance syndrome. *Atherosclerosis* 148: 141-149.
- Nadig, V. and Kotchen, T.A. (1997) Insulin resistance, blood pressure and cardiovascular disease. *Cardiol. Rev.* 5: 213-219.
- Nanjo, K., Sasaki, A., Seino, Y., Ito, C., Shima, K., Nonaka, K. and Kadowaski, T. (2002) Report of the committee on the classification and diagnosis criteria of diabetes mellitus. *Diabetes Res. Clin. Pract.* 55:65-85.
- Emilien, G., Maloteaux, J.M. and Ponchon, M. (1999) Pharmacological management of diabetes: recent progress and future perspective in daily drug treatment. *Pharmacol. Ther.* 81(1): 37-51.
- Goldwasser, I., Gefel, D., Gershonov, E., Fridkin, M. and shechter, Y. (2000) Insulin-like effects of vanadium: basic and clinical implications. *J. Inorg. Biochem.* 80: 21-25.
- Solomns, N.W. and Ruz, M. (1998) Trace element requirements in humans. An update. *J. Trace Elem. Exp. Med.* 11: 177-195.
- Willsky, G.R., Goldfine, A.B., Kostyniak, P.J., McNeill, J.H., Yang, L.Q. and Khan, H.R. (2001) Effect of vanadium (IV) compounds in the treatment of diabetes: in vivo and in vitro studies with vanadyl sulfate and bis (maltolato)oxovanadium (IV). *J. Inorg. Biochem.* 85: 33-42.
- Brichard, S.M. and Henquin, J.C. (1995) The role of vanadium in the management of diabetes. *Trends Pharmacol. Sci.* 16: 265-270.
- Dai, S. and McCneill, J.H. (1994) One-year treatment of non-diabetic and streptozotocin-diabetic rats with vanadyl sulfate did not alter blood pressure or hematological indices. *Pharmacol. Toxicol.* 74: 110-114.
- Shi, X., Flynn, D.C., Liu, K. and Dala, N. (1997) Vanadyl formation in the reduction of vanadate by glutathione reductase/NADPH and the role of molecular oxygen. *Ann. Clin. Lab. Sci.* 27 (6): 422-427.
- Bezerra, R.M., Veno, M., Silva, M.S., Tavares, D.Q., Carvalho, C.R. and Saad, M.J. (2001) A high-fructose diet induces insulin resistance but not blood pressure change in normotensive rats. *Braz. J. Med. Biol. Res.* 34 (9): 1155-1160.
- Storlien, L.H., Higgis, J.A., Thomas, T.C., Brown, M.A., Wang, H.Q. and Huang, X.F. (2000) Diet composition and insulin resistance action in animal models. *Br. J. Nutr.* 83 (Suppl1): S85-S90.
- Lee, M.K., Miles, P.D.G., Khoursheed, M., Gao, K.M., Moosa, A.R. and Olefsky, J.M. (1994) Metabolic effects of troglitazone on fructose-induced insulin resistance in the rat. *Diabetes* 43: 1435-1439.
- Southgate, D.A.T. (1995) Digestion and metabolism of sugars. *Am. J. Clin. Nutr.* 62 (suppl): 203S-211S.
- Randle, P.J. (1998) Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. *Diabetes Metab. Rev.* 14: 263-283.
- Grundy, M. (1999) Hypertriglyceridemia, insulin resistance, and metabolic syndrome. *Am. J. Cardiol.* 83: 25F-29F.
- Fantus, I.g. and Tsiani, E. (1998) Multifunctional actions of vanadium compounds on insulin signaling pathways: evidence for preferential enhancement of metabolic versus mitogenic effects. *Mol. cell Biochem.* 182: 109-119.
- Pandey, S.K., Anand-Srivastava, M.B. and Srivastava, A.K. (1998) Vanadyl sulfate-stimulated glycogen synthesis is associated with activation of phosphatidylinositol 3-kinase and is independent of insulin receptor tyrosine phosphorylation. *Biochemistry* 37: 7006-7014.