Low doses of vanadyl sulfate protect rats from lipid peroxidation and hypertriglyceridemic effects of fructose-enriched diet

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Abstract

Background: Insulin resistance, hyperinsulinemia and disturbances in lipid metabolism can be produced in healthy rats by feeding them a fructose-enriched diet. Vanadyl sulfate, an antidiabetic trace element, enhances insulin sensitivity in type 2 diabetic patients. The aim of this study was to determine the effect of vanadyl sulfate treatment (0.2 mg/ml in drinking water for 7 days) on plasma insulin, triglyceride concentration and plasma lipid peroxidation in rats that were fed a fructose-enriched diet that leads to insulin resistance. *Methods:* Male Wistar rats were divided into four groups: fructose-fed rats (FF); vanadyl sulfate treated-fructose fed treated rats (FV); control rats (C); and vanadyl sulfate-treated control rats (CV). Control and vanadyl sulfate-treated control rats were fed with standard laboratory chow. *Results:* High fructose feeding resulted in hyperinsulinemia and hypertriglyceridemia, and the plasma lipid peroxidation marker TBARS (thiobarbituric acid reactive substances) was significantly elevated. Administration of vanadyl sulfate was associated with significant normalization of plasma insulin and triglyceride levels. These rats also showed significantly lower TBARS than untreated, fructose-fed rats. *Conclusion:* We conclude that enhanced lipid peroxidation occurs in addition to hypertriglyceridemia in fructose-fed rats. It is suggested that lipid peroxidation associated with hypertriglyceridemia may be responsible for the pathologies induced by high fructose consumption. The plasma insulin level probably contributes to this increased peroxidation. Improved insulin action in fructose-fed vanadyl sulfate treated rats could be responsible for the amelioration of those abnormalities induced by fructose feeding. (Int J Diabetes Metab 14: 134-137, 2006)

Keywords: *Insulin resistance, Vanadyl sulfate, TBARS, Triglyceride, Fructose.*

Introduction

It has recently been shown that rats fed a fructose-enriched diet, that induces insulin resistance, develop oxidative stress. This oxidative stress improved when the diet was supplemented with antidiabetic agents.[1] An increasing number of studies have focused on the role of antidiabetic agents on lipid peroxidation.[2] Vanadium, an ultratrace element, is a new antidiabetic element,[3] that is used in experimental studies for the management of diabetes mellitus.[4] Because vanadium salts are used in glycemic homeostasis, their effects may depend strongly on changing insulin sensitivity in the liver and probably at the level of skeletal muscle.[5] The mechanisms of action of vanadium are not yet fully understood. The improvement in glucose uptake by the peripheral tissues could result from increased insulin binding to its membrane receptors, from the activation of post receptor metabolic pathways, as well as from a beneficial effect on lipid metabolism.[6] Although it was shown that vanadium compounds may increase lipid peroxidation,[7] it is unknown whether low doses of vanadyl ion (as vanadyl sulfate) in short term treatments lowers the lipid peroxidation marker TBARS when given in animal models of insulin resistance without fasting hyperglycemia, such as rats fed with high dosage of fructose.[1] Previous studies have shown that rats fed with a high-fructose diet developed insulin resistance and hypertriglyceridemia,[8] but not fasting hyperglycemia. This diet caused metabolic effects similar to those observed in syndrome X, in which insulin resistance, hypertension, and dyslipidemia are observed among patients with glucose intolerance[9]. In the light of previous studies that showed the beneficial effects of antidiabetic agents on lipid peroxidation, the goal of the present study was to evaluate the TBARS-lowering capability of vanadyl sulfate in Wistar rats with insulin resistance.

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Materials and Methods

Materials

Material used in this study included vanadyl sulfate ($VOSO_4$. $5H_2O$) (Merck Co, Germany). All other chemical reagents were of analytical grade.

Assays

Plasma vanadium concentration was measured in duplicate using a modified electrothermal atomic absorption spectrometric method with flameless atomic absorption Spectrometer (Perkin-Elmer model Zeeman 3030). Instrument settings and the graphite furnace program were: lamp current 0.5mA, slit width 0.7nm, wavelength 318.4nm, drying stage at 90-110°C for 10 second, ashing stage at 300-500°C for 10 second, pre atomization stage at 1200°C for 3

second, and atomization stage at 2650°C without delay.[10] Plasma TBARS was measured by a fluorometric method with Fluorescence Spectrometer (Perkin-Elmer L5-3P). Samples and standards for fluorescent analysis were recorded with excitation set at 530 nm and emission at 550 nm.[11] It is recommended that the sensitivity be set at high with a slit width of ≤ 5 nm. Plasma glucose and triglyceride determinations were performed on a RA 1000 autoanalyzer (Tecnichon, Co USA). Plasma insulin levels were determined in duplicate using a double-antibody radioimmunoassay technique and a gamma radiation counter (Model 1275 mini gamma, LKB Pharmacia Co).

Animals and Preparations

Male Wistar rats weighing 200-220 g (eight-week-old) were purchased from the Pasteur Institute (Tehran, Iran). Each group of rats was separately housed in a cage and had free access to water and food. Insulin resistance was induced in the rats by a fructose-enriched diet.[12] The diets are described in Table 1. At the beginning of the experiment, the rats were divided into four groups: fructose fed rats (FF, n=10); vanadyl treated-fructose fed rats (FV, n=10); control rats (n=8); as well as vanadyl-treated control rats (CV, n=8). The control and fructose-fed rats received tap water, and vanadyl treated-fructose fed and vanadyl treated control rats received tap water supplemented with 0.2mg/ml vanadyl sulfate freshly prepared every day. The treatment was carried out for 7 days. At the end of the treatment period (after an overnight fast), blood samples were collected from the retro-orbital venous plexus[13] into liquid lithium heparin as an anticoagulant (16 units/ml, 5 µl for 1 ml of blood)[14] and immediately centrifuged at 1,000g for 15 min. Plasma was removed and stored at -20° C until assayed.

Statistical data analysis

Results were expressed as mean ± SEM. Data were analyzed by independent-sample *t*-test. Significance level was set at $P < 0.05$.

Results

Insulin sensitivity and metabolic studies

The effects of fructose feeding and vanadyl sulfate consumption on plasma glucose, triglyceride, insulin, and vanadium concentrations in the different rat groups are shown in Table 2. Group FF developed significant insulin resistance (IR), as shown by its higher fasting insulin/fasting glucose ratio compared to the control group (P<0.001).

Table 1: Diet compositions of group C and group FF

	Control diet	FF diet
Starch	69	-
Fructose	-	66
Casein	20	20
Fat and Oil	10	12
Salts	0.5	1
Vitamin and Minerals	0.5	1

Data are given in grams/100 g of dry weight. C = Control, FF = Fructose-fed rats

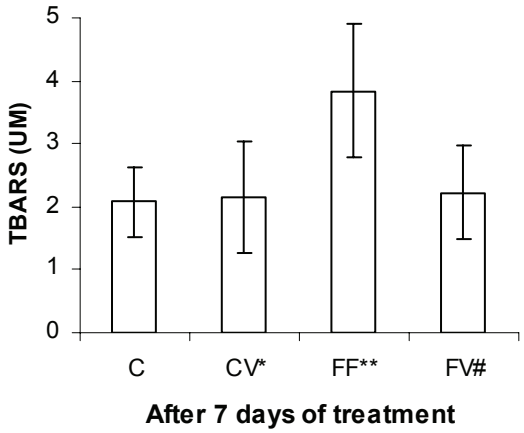

Figure 1: Plasma lipid peroxidation marker TBARS in different rat groups. Samples were collected after overnight food deprivation. Data are mean ± SEM of n = 8 for groups C and CV and n = 10 for groups FT and FV. C = control, CV = vanadyl-treated control, FF = fructose-fed, FV = vanadyl treated-fructose fed rats. *P> 0.05, compared to C rats. **P<0.01, compared to C rats. #P<0.01, compared to FF rats.

Insulin resistance improved significantly in the FV group. Group FF had much higher triglyceride levels than the control group (P<0.001). This parameter was significantly lower in the FV group. Fasting plasma glucose concentrations of the four groups were comparable (P>0.05) at the end of the treatment. Vanadyl sulfate did not change insulin and triglyceride levels of the CV in comparison with the control group. Significantly higher plasma vanadium was seen in groups given vanadyl sulfate.

Lipid peroxidation marker TBARS

The effects of fructose feeding and vanadyl sulfate consumption on plasma TBARS levels in the different rat groups are shown in figure 1. Group FF showed a significant increase in TBARS compared with the control group (P<0.01). TBARS decreased significantly in the FV group. Vanadyl sulfate did not change the TBARS in the CV group in comparison with the control group.

Discussion

The results of this study showed that vanadyl sulfate therapy increased insulin sensitivity in high fructose-fed rats and lowered plasma triglyceride levels. Simultaneously, vanadyl sulfate (as 75±18 µg/L vanadium concentration) decreased plasma TBARS levels. This improvement is indirect and is dependent on the effect of vanadyl on insulin sensitivity, since its TBARS lowering activity was not observed in the CV group.

Several reasons for insulin resistance in high fructose-fed rats have been reported. This phenomenon is believed to be related to the hypertriglyceridemic effect of fructose[15] Fructose feeding stimulates the hepatic production of triglycerides, both by promoting the re-esterification of

Table 2: IR and plasma glucose, insulin, vanadium, and triglyceride result in experimental rat groups (after 7 days).

	Control	CV	FF	FV
Glucose (mg/dl)	81±7	#78±8	#87±6	# 82±6
Triglyceride (mg/dl)	99±7	# 91±6	* 394±26	¶116±10
Insulin (pM)	83±3	# 74±4	*190±6	¶ 79±5
IR[1]	1.03±0.1	# 0.9±0.1	* 2.2 ±0.3	¶ 0.96±0.08
Vanadium (µg/L)	1.5±0.6	* 84±21	#1.7±0.8	* 75±18

Samples were collected after overnight food deprivation. Data are mean ± SEM, n = 8 for groups C and CV. n = 10 for groups FT and FV. [1]IR = insulin resistance (fasting insulin/fasting glucose).
*P< 0.001 compared to C rats. #P > 0.05, compared to C rats. ¶P<0.001, compared to FF rats.

circulating nonesterified fatty acids and by stimulation of *de novo* fatty acid synthesis.[16] Increased delivery of triglycerides or nonesterified fatty acids to muscle interferes with the utilization of glucose, through the operation of the Randle cycle,[17] impairing the insulin action. These findings conclusively demonstrate that enhancement of the sensitivity of target tissues to circulating insulin by vanadyl sulfate was related to the decrease in plasma triglyceride. Similar to insulin-sensitizer drugs,[15] vanadyl sulfate probably causes a decrease in plasma triglyceride, due to increased transcription of certain insulin sensitive genes, and promotes the expression of genes encoding lipoprotein lipase (LPL), a fatty acid transporter protein, thereby modulating the Randle glucose-fatty acid cycle. 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.[4-6,18] However, the cellular mechanism by which these effects are mediated remains poorly characterized. In addition, it should be remembered that high levels of circulating triglyceride interfere with insulin action at its receptor.[15] Consequently, our data demonstrate that improvement in physiological insulin action and prevention of insulin resistance by vanadyl ion is due, at least in part, to increasing insulin receptor binding. Although these findings are apparently not in agreement with those reported by others,[6] who showed that vanadium salts ameliorated glucose homeostasis in diabetic rats and several isolated cell types independent of the insulin receptor, it is suggested that vanadyl ion probably causes this effect through post receptor insulin signal transduction.

Previous studies have shown that a high-fructose diet markedly reduced the expression of antioxidant enzyme mRNA levels (e.g. catalase) in rat tissues,[19] and, on the other hand, impaired insulin action by causing defective intracellular antioxidant enzyme production.[20] Additionally, our results showed that the levels of the lipid peroxidation marker, TBARS significantly increased in the FF group but significantly decreased in the FV group, indicating a decrease in lipid peroxidation. Considering that lipid peroxidation was not decreased in the CV group, this confirmed that the effect of vanadyl sulfate in lowering plasma TBARS levels is related to improvement in insulin action. Although the mechanism of the antioxidant capability of low doses of vanadyl ion in the short term is not clearly understood, it might result from a beneficial effect on insulin action and increased expression of antioxidant enzyme mRNA levels.

In conclusion, the present study provides additional evidence that feeding with a high dosage of fructose leads to insulin resistance, hypertriglyceridemia, and oxidative stress. Finally, improved insulin action by vanadyl sulfate could be responsible for the amelioration of these abnormalities induced by fructose feeding.

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