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# Sodium Tungstate and Vanadyl Sulfate Effects on Blood Pressure and Vascular Prostanoids Production in Fructose-Overloaded Rats

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## Abstract

This study analyzes the effects of sodium tungstate and vanadyl sulphate in the fructose-overloaded rat, a model of metabolic syndrome. Fructose (9 weeks) increased blood pressure, triglycerydemia, glycemia, and reduced release of vasodilator prostaglandins (prostacyclin and prostaglandin E2 ) in the mesenteric vascular bed. Sodium tungstate prevented those alterations; meanwhile vanadyl sulfate only prevented the increase in glycemia. In conclusion, the present experiments showed that sodium tungstate is more effective than vanadyl sulfate for the treatment of experimental metabolic syndrome in rats.

**Keywords:** tungstate, vanadyl, fructose, prostanoids, triglycerides

## INTRODUCTION

The metabolic syndrome is a clustering of cardiovascular risk factors, whose major characteristics include insulin-resistance, hypertension, and lipid abnormalities (1). An oral fructose overload induces in the rat a mild hypertension associated with metabolic alterations such as hypertriglyceridemia and insulin-resistance resembling this syndrome (2–4). It is known that transition metals such as vanadium and tungsten have antidiabetic actions (5,6).

In this regard, sodium tungstate ameliorates dyslipemia and insulin-resistance in diet-induced obese rats (7). On the other hand, vanadium compounds showed glucose-lowering effects. In addition it has been shown that tungsten is also capable of reducing blood pressure (BP) in experimental models of hypertension such as Dahl (8) and spontaneously hypertensive rats (9).

Prostanoids, metabolites of arachidonic acid through the cyclooxygenases pathway, include vasoactive substances synthesized and released by the vessel wall. These agents have been implicated in an increased peripheral resistance, which has been postulated as one of the mechanisms involved in the fructose-induced hypertension. Moreover, an altered pattern of prostanoid release has been previously found in mesenteric vessels of experimental diabetic (10) and fructose-treated rats (11).

Therefore, the aim of the present study was to analyze the effects of the treatments with sodium tungstate and vanadyl sulfate on BP, metabolic parameters, and prostanoid release in aorta and mesenteric vascular bed from control and fructose-overloaded hypertensive rats.

## METHODS

Male, 6-week-old Sprague-Dawley rats weighing 170–210 g at the beginning of the study were used. The experiments were approved in advance by the local ethics committee on animal research. Animals were maintained in a room at 22 ± 2°C where the air was adequately recycled. All animals were fed with standard rodent diet (Asociación Cooperativas Argentinas) with the following composition (w/w): 20% proteins, 3% fat, 2% fiber, 6% minerals, and 69% starch and vitamins supplements.

Forty-two rats were randomly divided into six groups: controls (C), tap water to drink; n = 8; fructose-overloaded (F), fructose solution (10% w/v) to drink, n = 8; tungstate-treated (C-T), sodium tungstate solution (2 g/l w/v) to drink, n = 7; tungstate-treated fructose (F-T), both treatments, n = 7; vanadyl-treated (C-V), vanadyl sulfate solution (100 mg/l w/v) to drink, n = 6; and vanadyl-treated fructose (F-V), both treatments, n = 6. All treatments were administered for 9 weeks. The rats were acclimatized to the procedure of

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BP measurement at 10 AM, twice a week, for 2 weeks prior to being killed by decapitation. Indirect systolic blood pressure (SBP) was measured by means of a photoelectric tail-cuff connected to an amplifier (II TC model 47, Innovators in Instrumentation, Mays Landing, NJ, USA) in a series with an oscilloscope (Type 532, Tektronic Inc., Portland, OR, USA). In addition, rats were weighed before dietary manipulation and at the end of the study.

After 9 weeks, all experimental and control groups fasted for 5 h and then weighed. Blood samples were collected from the retro-orbital sinus and centrifuged at 4°C. Plasma glucose triglyceride and high-density lipoprotein (HDL) cholesterol levels were immediately measured by means of commercial kits (Wiener Glycemia and TG Color GPO/PAP AA, enzymatic methods, Wiener Labs, Rosario, Argentina) using spectrophotometric methods (automatic analyzer; Abbott Diagnostics, Abbott Park, IL, USA). After samples collection, the animals were sacrificed.

### Prostanoid Release Measurement

The thoracic aorta and the mesenteric bed of animals belonging to all groups were dissected and transferred to a Petri dish with Krebs solution (mM): NaCl 118, KCl 4.7, MgSO4 1.2, NaH2PO4 1.0, CaCl2 2.6, NaHCO3 25.0, glucose 11.1. The tissues were incubated in that solution for 60 min at 37°C. In order to measure the released prostanoids, at the end of the incubation period the media were acidified to pH 3.5 with 1 M formic acid and extracted three times with two volumes of chloroform. The chloroform fractions were pooled and evaporated to dryness. Reversed-phase high-performance liquid chromatography (HPLC) was carried out on a column (BBS Hypersil C18, Thermo Electron Co., Bellefonte, PA, USA). The solvent system was 1.7 mM H3PO4 67.2: acetonitrile 32.8 V/V. The flow rate was 1 ml.min-1 and UV absorption was measured at 218 nm. Dried samples were resuspended in 0.15 ml of the mobile phase and injected into the HPLC system.

Authentic standards of prostanoids: 6-keto prostaglandin (PG) F1$\alpha$ (stable metabolite of PGI2 or prostacyclin), PGE2, PGF2$\alpha$, and thromboxane (TX) B2 (stable metabolite of TXA2) (Sigma Chemical Co., Saint Louis, MO, USA) were run along with the samples and a bracket assay was performed to determine the amount of prostanoids. All values were corrected for recovery loss as determined by parallel standards. Results were expressed as nanograms of prostanoid per milligram of wet tissue weight.

### Statistical Analysis

All data are expressed as mean ± SEM. Intergroup comparisons were made by two-way analysis of variance (ANOVA). When necessary, the Bonferroni post-test was applied. A p value less than 0.05 was considered statistically significant.

## RESULTS

Nine weeks of fructose overload resulted in a significantly increased SBP (F vs. C, $p < 0.005$; Figure 1). Treatment with sodium tungstate prevented such an increase (F-T vs. F, $p < 0.001$). On the other hand, vanadyl sulfate produced a significant increase in SBP only in controls (C-V vs. C, $p < 0.005$).

The body weight and hypertrophy index (calculated as the ratio between the left ventricle weight and body weight) did not differ in any group (data not shown). Plasma data are shown in Figure 2. Panel A shows glycemia. Fructose treatment induced an increase in

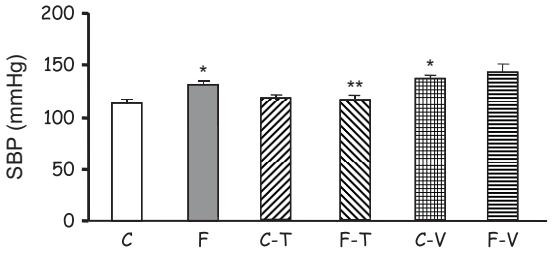


Figure 1. Systolic blood pressure (mm Hg) measured by the tail-cuff method in control (C, n = 8), fructose-overloaded (F, n = 8), sodium tungstate-treated (C-T, n = 7), fructose-tungstate (F-T, n = 7), vanadyl sulfate-treated (C-V, n = 6), and fructose-vanadyl rats (F-V, n = 6). Results are expressed as mean ± SEM. *$p < 0.005$ vs. C; **$p < 0.005$ vs. F.

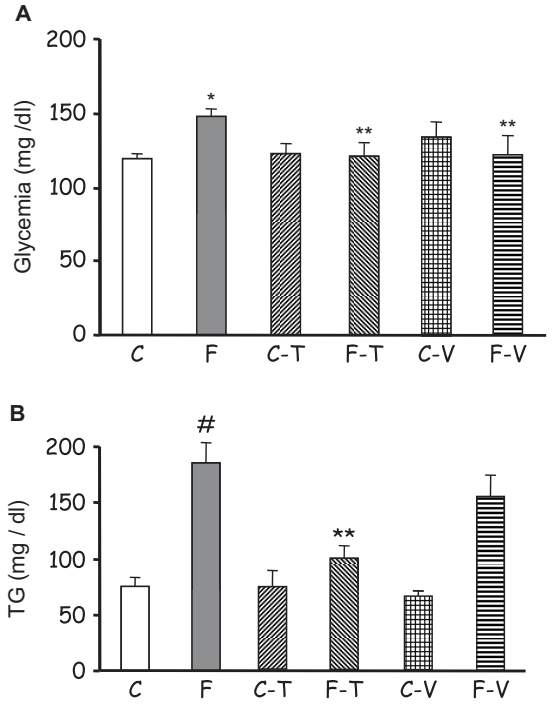


Figure 2. Glycemia (A) and triglyceridemia (B, mg/dl) measured by commercial kits in control (C, n = 8), fructose-overloaded (F, n = 8), sodium tungstate-treated (C-T, n = 7), fructose-tungstate (F-T, n = 7), vanadyl sulfate-treated (C-V, n = 6), and fructose-vanadyl rats (F-V, n = 6). Results are expressed as mean ± SEM. *:$p < 0.05$ vs. C; **$p < 0.05$ vs. F; #$p < 0.01$ vs. C.

plasma glucose levels (F vs C, $p < 0.05$). Sodium tungstate, as well as vanadyl sulfate, prevented such elevation (F-T vs. F, F-V vs. F, $p < 0.05$). As can be seen in Panel B, rats with fructose overload showed significantly higher triglyceridemia compared to the control group (F vs. C, $p < 0.01$). The sodium tungstate treatment prevented the increase in triglyceridemia in fructose overloaded animals (F-T vs. F, $p < 0.05$). This beneficial effect was not observed with vanadyl sulfate. Plasma HDL cholesterol levels were similar in all groups studied (data not shown).

6-keto PGF1$\alpha$ was the only prostanoid whose release was modified by 9 weeks of fructose overload in the thoracic aorta (C: 165±15 vs. F: 65±9 ng.mg wet tissue weight-1 ($p < 0.01$). Neither sodium tungstate nor vanadyl sulfate produced any alteration in prostanoid production in the thoracic aorta.

Figure 3 shows the vasodilator prostanoid production in the mesenteric vascular bed. As can be seen in Panel A and was previously reported (12), fructose overload for 9 weeks produced a significant reduction in 6-keto PGF1$\alpha$ release (F vs. C, $p < 0.005$). Sodium tungstate treatment prevented such an effect (F-T vs. F, $p < 0.005$). On the contrary, vanadyl sulfate was ineffective. Panel B shows a similar pattern for PGE2. Fructose also diminished PGE2 production (F vs. C, $p < 0.005$); sodium tungstate treatment prevented that reduction (F-T vs. F, $p < 0.001$). Meanwhile, vanadyl sulfate did not show any effect.

The vasoconstrictor prostanoid production in the mesenteric vascular bed is shown in Figure 4. No differences were found among groups in PGF2$\alpha$ (Panel A) or TXB2 release (Panel B).

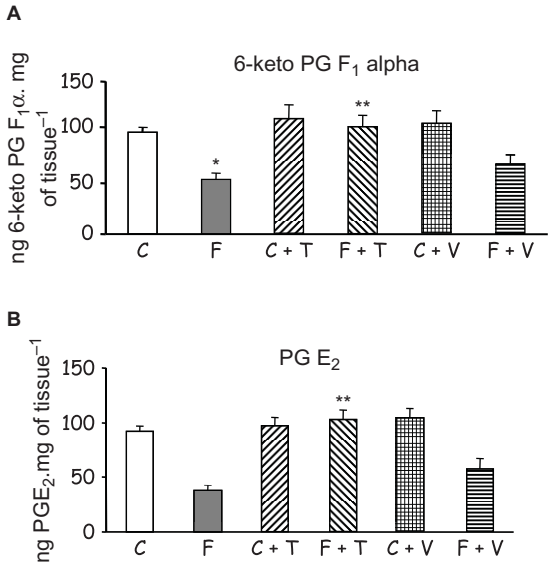


Figure 3. Release of 6-keto PGF1$\alpha$ and PGE2 (B, ng.mg tissue-1) by mesenteric vascular beds of control (C, n = 8), fructose-overloaded (F, n = 8), sodium tungstate-treated (C-T, n = 7), fructose-tungstate (F-T, n = 7), vanadyl sulfate-treated (C-V, n = 6), and fructose-vanadyl rats (F-V, n = 6). Results are expressed as mean ± SEM. *$p < 0.005$ vs. C; **$p < 0.005$ vs. F.

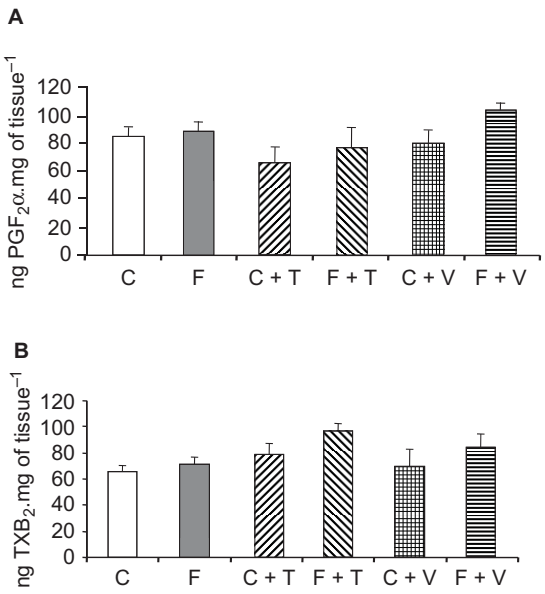


Figure 4. Release of PGF2α and TXB2 (B, ng.mg tissue-1) by mesenteric vascular beds of control (C, n = 8), fructose-overloaded (F, n = 8), sodium tungstate-treated (C-T, n = 7), fructose-tungstate (F-T, n = 7), vanadyl sulfate-treated (C-V, n = 6), and fructose-vanadyl rats (F-V, n = 6). Results are expressed as mean ± SEM.

## DISCUSSION

The present study shows that fructose overload increases SBP, glycemia, and triglyceridemia in the rat; meanwhile, sodium tungstate treatment prevents such increases. On the other hand, vanadyl sulfate lacks any effect on plasma triglycerides and BP. In addition, the later agent increases BP in control animals; meanwhile, showed a tendency to augment it in fructose-treated rats.

Regarding vascular prostanoid production, as previously reported by our laboratory (13), 9 weeks of fructose overload diminished the vasodilators prostacyclin and PGE2 in the mesenteric vascular bed. Sodium tungstate prevents such decreases; meanwhile, vanadyl sulfate lacked any effect on these prostanoids.

Banhot, McNeill, and Bryer-Ash (13) and Al-Awwadi et al. (14) found that V prevents hypertension in fructose rats in opposition to our study. On the other hand, Al Awwadi (14), in accordance with our results, found a BP increase in vanadium-treated control animals. Regarding tungstate treatment, our findings are also similar to those of Al-Awwadi et al. (14) in its preventative effect on the fructose-induced hypertension. The observed differences could be attributed to different ways of fructose administra-tion (diet vs. drinking water) and duration of treatment (9 weeks in our case, 6 weeks for Al-Awwadi et al. (14) and 4 weeks for Bhanot et al. (14)).

Nagareddy, Vasudevan, and McNeill (15) reported beneficial effects of tungstate treatment on cardiac performance in diabetic rats. In addition, it is a well-known fact that tungstate is an effective inhibitor of xanthine oxidase, an enzyme involved in the formation of free radicals (16). Moreover, in this model an increased level of oxidative stress has been described (17) and in this condition the excessive production of reactive oxygen species (ROS) can inhibit nitric oxide (NO) and prostacyclin production (18) with a concomitant elevation in BP.

On the other hand, the vanadyl sulfate hypertensive effect observed in control rats could be attributed, at least in part, to its contractile properties described in the isolated aorta (19). In addition, vanadate was found to augment free intracellular Ca2+ in smooth muscle cells, thereby promoting an increase in vascular tone and contractility (20). Zhuowei et al. (21) also found that vanadyl sulfate causes pulmonary artery constriction in rabbits through an inhibition of endothelial NO synthesis.

Regarding the effects of tungstate on plasma triglycerides, our results are in accordance with those of Muñoz et al. (22), who found that tungstate decreased serum triglyceride concentrations in Zucker diabetic rats. As far as we know, this is the first study that deals with the effects of vanadyl sulfate on triglyceridemia in fructose-overloaded rats. We did not find any effect neither in controls nor in treated animals.

Concerning its actions on plasma triglycerides in related models, reports are controversial. In a recent study performed in patients with impaired glucose tolerance Jacques-Camarena et al. (23) found increased levels of triglycerides after 4 weeks of administration of vanadyl sulfate. Conversely, Willsky et al. (24) reported that 4 weeks of treatment with this agent normalized elevated triglyceridemia in streptozotocin-diabetic rats.

Vanadyl sulfate treatment did not modify prostanoid release by the mesenteric vascular bed. On the other hand, sodium tungstate treatment was able to prevent the impairment in the production of PGI2 and PGE2 induced by fructose overload, which did not modify TX release, resulting in a decrease in the prostacyclin/TX ratio. A diminished ratio is associated with the progression of vascular complications in diabetes, a pathology related to metabolic syndrome. This has been reported in rat mesenteric vascular bed (10) as well as in diabetic patients (25).

The mechanisms involved in this effect remain to be elucidated. Nevertheless, as it was previously mentioned, an increased production of ROS has been reported in the fructose overloaded rat model (17). Moreover, in this condition such overproduction can inhibit prostacyclin release (16). On the other hand, it has been shown that tungstate inhibits xanthine oxidase, an enzyme involved in the formation of free radicals (18). Taken together, these observations could explain, at least in part, the alterations of vascular prostanoid production in fructose overloaded animals and its prevention by sodium tungstate.

In conclusion, the present experiments showed that, in our working conditions, sodium tungstate appears to be more effective than vanadyl sulfate for the treatment of experimental metabolic syndrome in rats. The increase in BP observed in this model could be derived from an augmented peripheral resistance due, at least in part, to a reduction of the vascular release of vasodilator prostanoids. Sodium tungstate was able to prevent such alteration; meanwhile, vanadyl sulfate lacked any effect.

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