Autonomic & Autacoid Pharmacology

Autonomic and Autocoid Pharmacology
2013, 33, 43–48

Sodium molybdate prevents hypertension and vascular prostanoid imbalance in fructose-overloaded rats

H. A. Peredo, V. Andrade, A. S. Donoso, H. J. Lee & A. M. Puyó

Cátedra de Anatomía Humana (macro y microscópica) and INFIBIOC, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires; CONICET, Junín 956, 1113, Buenos Aires, Argentina

Correspondence:
H. A. Peredo

Summary

1. Fructose (F) overload produces elevated blood pressure (BP), hyperglycaemia, hypertriglyceridemia and insulin resistance, resembling human metabolic syndrome. Previously, we found altered vascular prostanoid (PR) production in this model.
2. Sodium molybdate (Mo), as well as sodium tungstate, causes insulin-like effects and normalizes plasma glucose levels in streptozotocin-treated diabetic rats. We studied the effects of Mo on BP, metabolic parameters and release of PR from the mesenteric vascular bed (MVB) in F-overloaded rats.
3. Four groups of male Sprague-Dawley rats were analysed: Control, tap water to drink; F, F solution 10% W/V to drink; CMo, Mo 100 mg kg day^{-1} and FMo, both treatments. After 9 weeks, the animals were killed and MVBs removed and the released PRs measured.
4. F increased BP, glycemia, triglyceridemia and insulinemia. Mo treatment prevented the increases in BP and glycemia, but did not modify triglyceridemia or insulinemia. In addition, Mo decreased BP in controls.
5. Prostaglandins (PG) F_2alpha and E_2, PG 6-ketoF_1alpha and thromboxane (TX) B_2, as well as inactive metabolites of prostacyclin (PGI_2) and TXA_2 were detected. F decreased the production of vasodilator PRs PGI_2 and PGE_2 in MVB. Mo prevented these alterations and increased PGE_2 in controls. Vasoconstrict or PRs PGF_2alpha and TXA_2 release was not modified.
6. Mo treatment, beyond its known lowering effect on glycemia, prevents the reduction in the vascular release of vasodilator PR observed in this model. This could be one of the mechanisms by which Mo avoids the increase in BP caused by F overload in the rat.

Keywords: molybdate, fructose, hypertension, prostanoids, metabolic syndrome

Introduction

Transition metal compounds such as vanadium, tungsten and molybdenum act as insulin mimetics decreasing plasma glucose levels and have been proposed as a possible treatment for diabetes mellitus. Molybdate compounds normalize plasma glucose levels in streptozotocin- or alloxan-diabetic rats (Panneerselvam & Govindasamy, 2004; Zeng *et al.*, 2008). Molybdenum is the only second-row transition metal that is required by most living organisms, being available to biological systems through the solubility of molybdate salts in water. Moreover, molybdenum-containing enzymes are found in all aerobic organisms and are divided into three families: xanthine oxidase, sulphite oxidase and nitrate reductase (Hille, 2002).

In developed countries, the prevalence of metabolic syndrome, which major characteristics include insulin resistance, hypertension and lipid abnormalities (Reaven, 1988; Alberti *et al.*, 2006), contributes to the increased risk of type 2 diabetes and cardiovascular disease. The fructose-fed rat is an experimental model of acquired systolic hypertension that resembles the human metabolic syndrome (Hwang *et al.*, 1987; Tran *et al.*, 2009). In previous works with this model (Puyó *et al.*, 2009), we found alterations in the vascular production of prostanoids, metabolites of cyclo-oxygenation of arachidonic acid. Such alterations could modify peripheral resistance, which increase is one of the possible mechanisms of fructose-induced hypertension. Moreover, we also found that sodium tungstate and vanadyl sulphate have different effects on blood pressure,

Autonomic and Autocoid Pharmacology
2013, 33, 43–48

metabolic parameters and vascular prostanoid production in fructose-overloaded rats (Peredo *et al.*, 2010). It has been previously reported by Güner *et al.* (2001) that sodium molybdate prevented blood pressure and metabolic alterations in a similar experimental model.

The aim of this study was to analyse the possible effects of sodium molybdate treatment on blood pressure, metabolic parameters and prostanoid release in vascular preparations from control as well as fructose-overloaded rats.

Methods

Male 6-week-old Sprague-Dawley rats weighing 180–220 g at the beginning of the study were used. Animals were maintained in a room at 22 ± 2 °C where the air was adequately recycled, and they were fed with standard rodent diet (Asociación Cooperativas Argentinas) with the following composition (w/w): 20% proteins, 3% fat, 2% fibre, 6% minerals and 69% starch and vitamins supplements. Twenty-two rats were randomly divided into four groups: controls (C), tap water to drink, $n = 6$; fructose-overloaded (F), fructose solution (10% w/v) to drink, $n = 6$; sodium molybdate-treated (CMo), (100 mg kg day^{-1}) to drink, $n = 6$, Mo-treated fructose (FMo), both treatments. All dietary modifications were applied for 9 weeks. The rats were acclimatized to the procedure of blood pressure measurement at 10.00 A.M., twice a week, for 2 weeks prior to sacrifice. Indirect systolic blood pressure was measured by means of a photoelectric tail cuff connected to an amplifier (II TC model 47; Innovators in Instrumentation, Landing, NJ, USA) in 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.

At the end of the treatments, all experimental and control groups were fasted for 5 h and weighed. Blood samples were collected from the retro-orbital sinus and centrifuged at 4 °C. Plasma triglyceride levels were immediately measured by means of commercial kits (TG Color GPO/PAP AA, Wiener Labs, Rosario, Santa Fé, Argentina) using spectrophotometric methods; plasma glucose by a blood glucose metre (Accu-Chek, Roche Diagnostics GmbH, Mannheim, Germany) and insulin by ELISA (Millipore Corporation, Billerica, MA, USA). After collection of samples, the animals were sacrificed and the hearts were dissected and weighed to calculate the hypertrophy index.

The thoracic aorta and the mesenteric bed of animals of all groups were dissected and transferred to a Petri dish with Krebs' solution (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, NaH_2PO_4 1.0, $CaCl_2$ 2.6, $NaHCO_3$ 25.0, glucose 11.1. The tissues were incubated in that solution for 60 min at 37 °C. To measure the released prostanoids, at the end of the incubation period, 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 HPLC was carried out on a column (BBS Hypersil C18, Thermo Electron Co., Bellefonte, PA, USA). The solvent system was 1.7 mM H_3PO_4 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) $F_1\alpha$ (stable metabolite of PGI_2 or prostacyclin), PGE_2, $PGF_2\alpha$ and thromboxane (TX) B_2 (stable metabolite of TXA_2) (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.

All data are expressed as mean ± SEM. Intergroup comparisons were made by one-way analysis of variance (ANOVA). When necessary, the Tukey's *post hoc* test was applied. A P value of less than 0.05 was considered statistically significant.

Results

Body weight, the cardiac hypertrophy index (as the wet heart weight to body weight ratio) and plasma data are shown in Table 1. Rats with fructose overload showed significantly higher levels of glycemia ($P < 0.02$), triglyceridemia ($P < 0.005$) and insulinemia ($P < 0.02$) compared with control group. Sodium molybdate treatment prevented the increase in plasma glucose ($P < 0.002$), compared with the fructose group. Plasma triglycerides and insulin levels remained elevated in the fructose plus sodium molybdate group. Cardiac hypertrophy index and body weight did not differ in any group.

Figure 1 shows systolic blood pressure levels in all groups. Rats with fructose overload showed significantly higher levels ($P < 0.001$). Sodium molybdate treatment prevented such increase and also diminished systolic blood pressure in control animals ($P < 0.02$).

As previously reported (Peredo *et al.*, 2008), 6-keto $PGF_1\alpha$ was the only prostanoid where release was modified by 9 weeks of fructose overload in the thoracic aorta (C: 165 ± 15 *vs.* F: 65 ± 9 ng mg wet tissue $weight^{-1}$; $P < 0.001$). Sodium molybdate did not produce any alteration

Autonomic and Autocoid Pharmacology
2013, 33, 43–48

Table 1 Body weight, cardiac hypertrophy index (as the wet heart weight-to-body weight ratio) and metabolic parameters of control, fructose-overloaded, sodium molybdate-treated and fructose-molybdate rats.

	Body weight (gr)	Cardiac hypertrophy index	Triglyceridemia (mg dl^{-1})	Glycemia (mg dl^{-1})	Insulinemia (ng ml^{-1})
Control (n = 6)	487 ± 9	0.0027 ± 0.0001	47 ± 4	127 ± 6	1.9 ± 0.3
Fructose (n = 6)	464 ± 17	0.0030 ± 0.0002	163 ± 15 $P < 0.005$ *vs.* C	156 ± 10 $P < 0.02$ *vs.* C	4.5 ± 0.8 $P < 0.02$ *vs.* C
Control + Molybdate (n = 6)	468 ± 30	0.0029 ± 0.0002	64 ± 5	133 ± 14	2.6 ± 0.3
Fructose + Molybdate (n = 5)	460 ± 23	0.0029 ± 0.0001	160 ± 10 $P < 0.0001$ *vs.* CMo	100 ± 5 $P < 0.002$ *vs.* F	4.8 ± 1.0

Results are expressed as mean ± SEM.

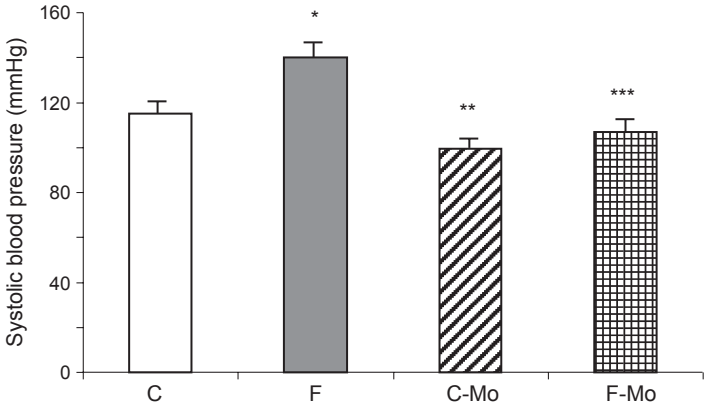

Figure 1 Systolic blood pressure (mm Hg) measured by the tail-cuff method in control (C, mm n = 8), fructose-overloaded (F, n = 6), sodium molybdate-treated (C + Mo, n = 6) and fructose-molybdate rats (F + Mo, n = 5). *$P < 0.001$ *vs.* C; **$P < 0.02$ *vs.* C; ***$P < 0.02$ *vs.* F. Results are expressed as mean ± SEM.

in prostanoid production in this vascular preparation.

Fructose overload decreased the production of the vasodilator prostanoids PGI_2 (Fig. 2) and PGE_2 (Fig. 3) in the mesenteric vascular bed ($P < 0.001$). Sodium molybdate prevented these alterations ($P < 0.02$ and < 0.001 respectively). Moreover, the treatment increased PGE_2 release also in control animals ($P < 0.05$, Fig. 3). The release of vasoconstrictor prostanoids $PGF_2\alpha$ and TXA_2 was not modified in any groups (data not shown).

The prostacyclin/thromboxane release ratio was reduced by fructose overload both in aorta and mesenteric bed ($P < 0.001$). In the latter vascular preparation, sodium molybdate administration showed a tendency to prevent that alteration which did not reach statistical significance. On the other hand, the treatment increased the ratio in control animals ($P < 0.02$, Fig. 4).

Discussion

The present study shows that sodium molybdate prevented the increases in glycemia and systolic blood pressure produced by fructose overload; meanwhile, triglyceridemia and insulinemia remained unchanged. In addition, this agent also reduced blood pressure in control animals. Regarding vascular prostanoid production, the treatment prevented the decrease in vasodilator metabolites induced by fructose in the mesenteric bed. Moreover, in control animals, PGE_2 release was increased, and the PGI_2/TXA_2 ratio, an indicator of vascular dysfunction, was also improved.

As far as we know, the only work that previously explored the effect of sodium molybdate treatment on fructose-overloaded rats is that of Güner *et al.* (2001). In agreement with our results, these authors found that the treatment prevented the elevations in blood pressure and plasma glucose levels. They also reported a similar prevention of hypertriglyceridemia and hyperinsulinemia, but these effects were not observed in our study. These differences could be attributed to the fact that they used Wistar instead of Sprague-Dawley rats and treated the animals for a longer period.

In a previous work of our laboratory (Peredo *et al.*, 2010), we analysed the treatment with

Autonomic and Autocoid Pharmacology
2013, 33, 43–48

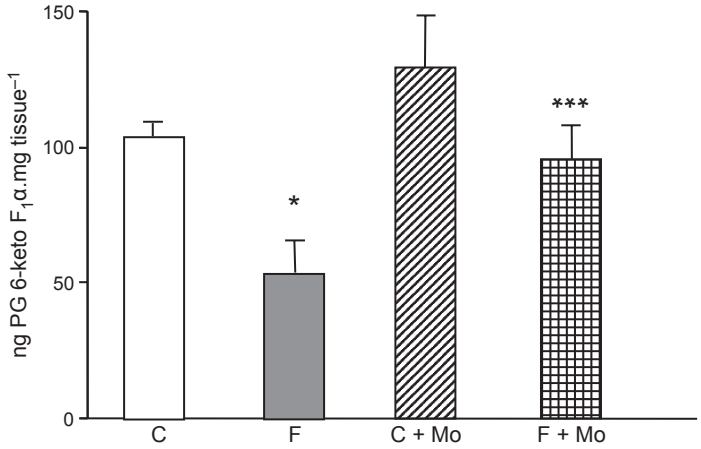

Figure 2 Release of 6-keto $PGF_1\alpha$ (ng mg tissue^{-1}) by mesenteric vascular beds of control (C, $n = 8$), fructose-overloaded (F, $n = 6$), sodium molybdate-treated (C + Mo, $n = 6$), and fructose-molybdate (F + Mo, $n = 5$). *$P < 0.001$ *vs.* C; ***$P < 0.02$ *vs.* F. Results are expressed as mean ± SEM.

other transition metal compounds (such as tungsten and vanadium derived salts) in this experimental model, but their effects on blood pressure and metabolic parameters differed from those obtained with sodium molybdate. Meanwhile, sodium tungstate lowers not only blood pressure but also glycemia and triglyceridemia as well; vanadyl sulphate had a positive effect on plasma glucose levels, but caused hypertension in control rats and lacked any effect on blood pressure and triglycerides plasma levels in fructose-overloaded animals.

More data are available about transition metal compounds on diabetes, especially for their known lowering effects on plasma glucose. It is well established that trace elements are altered in diabetic patients as compared with healthy subjects (Aguilar *et al.*, 2007). Among the metal containing drugs, the vanadium complexes have been the most tested as potential therapeutic agents for oral treatment of type 2 diabetes mellitus (Kurt *et al.*, 2011; Thompson *et al.*, 2009), but a range of other metal compounds has also been tried as possible alternative antihyperglycaemic drugs.

Molybdenum and vanadium compounds present some similarities of action. For instance, both are able to inactivate glycogen synthase (Thompson *et al.*, 2004) and stimulate glucose uptake in rat adipocytes in the presence of H_2O_2 (Chaves *et al.*, 2010). In addition, they also enhance insulin secretion by means of increasing cultured islets' function (Mohseni Salehi Monfared & Pournourmohammadi, 2010). In spite of the fact that their glucose lowering mechanisms are alike in diabetic rats, we did not find any effect on the hypersecretion of insulin in fructose rats.

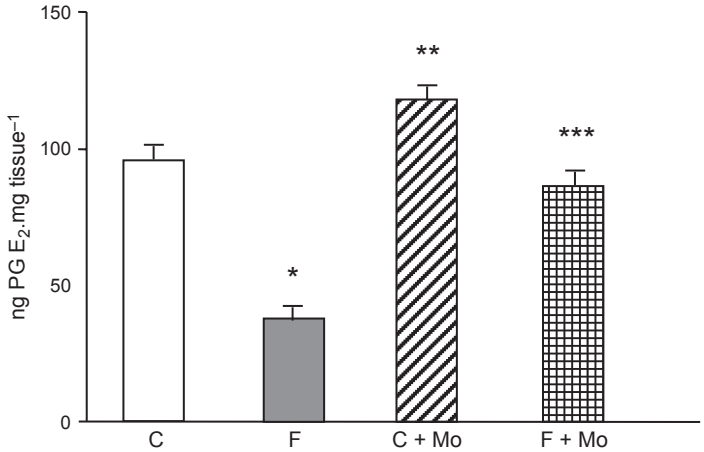

Figure 3 Release of PGE_2 (ng mg tissue^{-1}) by mesenteric vascular beds of control (C, $n = 8$), fructose-overloaded (F, $n = 6$), sodium molybdate-treated (C + Mo, $n = 6$), and fructose-molybdate (F + Mo, $n = 5$). *$P < 0.001$ *vs.* C; **$P < 0.05$ *vs.* C; ***$P < 0.001$ *vs.* F. Results are expressed as mean ± SEM.

Autonomic and Autocoid Pharmacology
2013, 33, 43–48

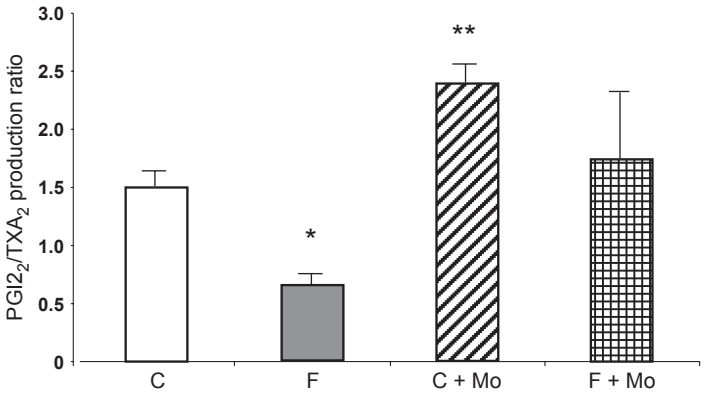

Figure 4 PGI_2/TXA_2 release ratio by mesenteric vascular beds of control (C, $n = 8$), fructose-overloaded (F, $n = 6$), sodium molybdate-treated (C + Mo, $n = 6$), and fructose-molybdate (F + Mo, $n = 5$). $^*P < 0.001$ *vs.* C; $^{**}P < 0.02$ *vs.* C. Results are expressed as mean ± SEM.

Regarding the control of plasma lipid levels, it was reported that molybdate diminished non-esterified fatty acids (Ozcelikay *et al.*, 1996) as well as triglycerides, phospholipids and cholesterol in diabetic rats (Panneerselvam & Govindasamy, 2004). On the other hand, when we analysed triglyceride levels in fructose-treated animals, no improvement was observed. A possible explanation to this differential effect between both experimental models could be derived from the much higher levels of triglycerides found in rats with metabolic syndrome compared with those in diabetic animals.

The main finding in our study was the effect of sodium molybdate on blood pressure. That transition metal salt not only improved hemodynamic parameters in fructose-overloaded rats but also diminished blood pressure in control animals. This latter effect could be related, at least in part, to the increased release of the vasodilator prostanoid PGE_2 and the improvement of the PGI_2/TXA_2 ratio in that group.

The endothelial dysfunction present in these animals is partly derived from a previously reported reduction in the release of nitric oxide (Tran *et al.*, 2009; Carranza *et al.*, 2011) and the prostanoids PGI_2 and PGE_2 (Peredo *et al.*, 2010). Moreover, the prevention of the decrease in PGI_2 production in fructose-overloaded animals could be also involved in the reduction in blood pressure, given the well-known fact that one of the mechanisms that elevate that parameter in this model is an enhancement of vascular resistance.

Fructose overload is also associated with an increase in oxidative stress (Rebolledo *et al.*, 2008; Carranza *et al.*, 2011), and the excessive production of reactive oxygen species (ROS) has been reported to inhibit nitric oxide and prostacyclin release in endothelial cells (Du *et al.*, 2006); such reduction can cause an increase in blood pressure. Some antioxidant diet-derived compounds act through enzymatic mechanisms, and the activity of antioxidant enzymes depends on the intake of trace metals such as molybdenum (Chan *et al.*, 1998; Hille *et al.*, 1998).

In conclusion, molybdate treatment, beyond its known lowering effect on glycemia, prevents the reduction in the vascular release of vasodilator prostanoids observed in this model. This could be one of the mechanisms by which sodium molybdate avoids the increase in blood pressure caused by fructose overload in the rat. Moreover, molybdate also reduces blood pressure in controls, an effect that also could be attributable, at least in part, to modifications in the vascular prostanoid release profile.

Acknowledgments

This study was supported by grants from the Secretaría de Ciencia y Técnica of the Universidad de Buenos Aires, code 121 and Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) PICT 07/00994. The authors wish to thank to Jennifer S. Borroni and Yanina A. Santander for their excellent technical assistance.

References

AGUILAR, M.V., SAAVEDRA, P., ARRIETA, F.J., MATEOS, C.J., GONZÁLEZ, M.J., MESEGUER, I. & MARTÍNEZ-PARA, M.C. (2007). Plasma mineral content in type-2 diabetic patients and their association with the metabolic syndrome. *Ann. Nutr. Metab.*, **21**, 402–406.

ALBERTI, K.G.M.M., ZIMMET, P. & SHAW, J. (2006). Metabolic syndrome-a new world-wide definition. A consensus statement from the Federation, International Diabetes. *Diabet. Med.*, **23**, 469–480.

CARRANZA, A., LITTERIO, M.P., PRINCE, P.D., MAYER, M.A., INGARAMO, P.I., RONCO, N.T., PEREDO, H.A., PUYÓ, A.M. & GALLEANO, G. (2011).

Autonomic and Autocoid Pharmacology
2013, 33, 43–48

Lipopolysaccharide (LPS) induction of nitric oxide synthase-2 & cyclo-oxygenase-2 is impaired in fructose overloaded rats. *Life Sci.*, **88**, 307–313.

CHAN, S., GERSON, B. & SUBRAMANIAM, S. (1998). The role of copper, molybdenum, selenium & zinc in nutrition & health. *Clin. Lab. Med.*, **18**, 673–685.

CHAVES, S., JELIC, R., MENDONÇA, C., CARRASCO, M., YOSHIKAWA, Y., SAKURAI, H. & SANTOS, M.A. (2010). Complex of hydroxyl(thio)pyrone & hydroxyl(thio)pyridinone with Zn(II) & Mo(IV) Thermodynamic stability & insulin-mimetic activity. *Metallomics*, **2**, 220–227.

DU, X., EDELSTEIN, D., OBICI, S., HIGHAM, N. & ZOU, M.-H. (2006). Insulin resistance reduces arterial prostacyclin synthase & eNOS activities by increasing endothelial fatty acid oxidation. *J. Clin. Invest.*, **116**, 1071–1080.

GÜNER, S., TAY, A., ALTAN, V.M. & ÖZCELIKAY, A.T. (2001). Effect of sodium molybdate on fructose-induced hyperinsulinemia & hypertension in rats. *Trace Elem. Electrol.*, **18**, 39–46.

HILLE, R. (2002). Molybdenum and tungsten in biology. *Trends Biochem. Sci.*, **27**, 360–367.

HILLE, R., RETEY, J., BARTLEWSKI-HOF, U. & REICHENBECHER, W. (1998). Mechanistic aspects of molybdenum-containing enzymes. *FEMS Microbiol. Rev.*, **22**, 489–501.

HWANG, Y., HO, H., HOFFMAN, B.B. & REAVEN, G.M. (1987). Fructose-induced insulin resistance and hypertension in rats. *Hypertension*, **10**, 512–516.

KURT, O., OZDEN, T.Y., OSZOY, N., TUNALI, S., CAN, A., AKEV, N. & YANARDAQ, R. (2011). Influence of vanadium supplementation on oxidative stress factors in the muscle of STZ diabetic rats. *Biometals*, **24**, 943–949.

MOHSENI SALEHI MONFARED, S.S. & POURNOURMOHAMMADI, S. (2010). Teucrium polium complex with molybdate enhance cultured islets secretory function. *Biol. Trace Elem. Res.*, **133**, 236–241.

OZCELIKAY, A.T., BECKER, D.J., ONGEMBA, L.N., POTTIER, A.M. & HENQUIN, J.C., BRICHARD, S.M. (1996). Improvement of glucose & lipid metabolism in diabetic rats treated with molybdate. *Am. J. Physiol.*, **270**, E344–E352.

PANNEERSELVAM, S.R. & GOVINDASAMY, S. (2004). Effect of sodium molybdate on the status of lipids, lipid peroxidation & antioxidant systems in alloxan-induced diabetic rats. *Clin. Chim. Acta*, **345**, 93–98.

PEREDO, H.A., MAYER, M.A., CARRANZA, A. & PUYÓ, A.M. (2008). Pioglitazone & losartan prevent hypertension & hypertriglyceridemia & modify vascular prostanoids in fructose-overloaded rats. *Clin. Exp. Hypertens.*, **30**, 159–169.

PEREDO, H.A., ZABALZA, M., MAYER, M., CARRANZA, A. & PUYÓ, A.M. (2010). Sodium tungstate & vanadyl sulfate effects on blood pressure & vascular prostanoid production in fructose-overloaded rats. *Clin. Exp. Hypertens.*, **32**, 453–457.

PUYÓ, A.M., ZABALZA, M., MAYER, M., CARRANZA, A. & PEREDO, H.A. (2009). Time-course of vascular prostanoid production in the fructose-hypertensive rat. *Auton. Autacoid Pharmacol.*, **29**, 135–139.

REAVEN, G.M. (1988). Banting Lecture 1988: role on insulin resistance in human disease. *Diabetes*, **37**, 1597–1607.

REBOLLEDO, O.R., MARRA, C.A., RASCHIA, A., RODRIGUEZ, S. & GAGLIARDINO, J.J. (2008). Abdominal adipose tissue: early metabolic dysfunction associated to insulin resistance & oxidative stress induced by an unbalanced diet. *Horm. Metab. Res.*, **40**, 794–800.

THOMPSON, K.H., CHILES, J., YUEN, V.G., TSE, J., MC NEILL, J.H. & ORVIG, C. (2004). Comparison of anti-hyperglycemic effect amongst vanadium, molybdenum & other metal maltol complexes. *J. Inorg. Biochem.*, **98**, 883–890.

THOMPSON, K.H., LICHTER, J., LEBEL, C., SCAIFE, M.C., MCNEILL, J.H. & ORVIG, C. (2009). Vanadium treatment of type-2 diabetes: a view to the future. *J. Inorg. Biochem.*, **103**, 554–558.

TRAN, L.T., YUEN, V.G. & MCNEILL, J.H. (2009). The fructose-fed rat: a review on the mechanisms of fructose-induced insulin resistance & hypertension. *Mol. Cell. Biochem.*, **332**, 145–159.

ZENG, C., HOU, G., DICK, R. & BREWER, G.J. (2008). Tetrathiomolybdate is partially protective against hyperglycaemia in rodent models of diabetes. *Exp. Biol. Med.*, **233**, 1021–1025.

(Received 26 March 2013
Accepted 07 May 2013)