Aggravation by vanadium of magnesium deficiency in STZ-induced diabetic rats

M.C. Bermúdez-Peña3, C. López-Chaves, J. Llopis1, F. Guerrero-Romero3, M. Montes-Bayón2, A. Sanz-Medel2, C. Sánchez-González1

1 Institute of Nutrition and Food Technology and Department of Physiology, Campus Cartuja, University of Granada, E-18071 Granada, Spain; 2 Department of Physical and Analytical Chemistry, University of Oviedo, Oviedo, Spain; 3 Biomedical Research Unit, Mexican Social Security Institute, Victoria de Durango, Mexico

Correspondence: Cristina Sánchez-González. Instituto de Nutrición y Tecnología de Alimentos. Centro de Investigaciones Biomédicas Lab. 115. Universidad de Granada, Parque Tecnológico de la Salud 18100 Armilla, Granada (Spain)
<crissg@ugr.es>

ABSTRACT. This study examined changes in the metabolism of magnesium (Mg), and related serum parameters, following treatment with vanadium (V) in streptozotocin-diabetic rats. Over a period of five weeks, four groups were examined: control, diabetic, diabetic-treated with 1 mg V/day or 3 mg V/day. The V was supplied in drinking water as bis(maltolato)oxovanadium(IV). The Mg levels were measured in food, faeces, urine, serum, muscle, kidney, liver, spleen, heart and femur. Albumin, uric acid, urea, total-cholesterol, LDL-cholesterol, triglycerides, aspartate-aminotransferase and alkaline-phosphatase were determined in serum. In the diabetic group, Mg retained and Mg content in serum and femur decreased, while levels of uric acid, urea, total-cholesterol, LDL-cholesterol, triglycerides and alkaline-phosphatase and aspartate-aminotransferase activity increased compared with control rats. In the diabetic group treated with 1 mg V/day, Mg retained, serum levels of Mg, urea and triglycerides, and alkaline-phosphatase activity remained unchanged, while levels of uric acid, total-cholesterol and LDL-cholesterol increased and the Mg content in femur and aspartate-aminotransferase activity decreased compared with the diabetic untreated group. In the diabetic rats treated with 3 mg V/day, food intake and glycaemia were normal. In this group, Mg content in serum, kidney and femur, levels of urea and aspartate-aminotransferase and alkaline-phosphatase activity decreased, whereas LDL-cholesterol increased, uric acid and total-cholesterol levels remained unchanged in comparison with untreated diabetic rats. In conclusion, although treatment with 3 mg V/day normalised the glycaemia, the hypomagnesaemia and tissue depletion of Mg seen in the diabetic rats, caused by the treatment with V, could have partially contributed to the fact that V did not normalise other serum parameters altered by the diabetes.

Key words: vanadium, magnesium, diabetes, interactions, blood parameters
Some complexes of vanadium (V) have been shown to possess hypoglycaemic effects, stimulating autophosphorylation of the insulin receptors and increasing the activity of tyrosine kinase, favouring the translocation of the glucose transporter GLUT 4 [1, 2]. The V complex, bis(maltolato)oxovanadium (IV) (BMOV), is known to be more effective than inorganic V as a glucose-lowering agent [1].

Although some V compounds are currently undergoing human clinical trials [1], many aspects remain to be determined, such as its interactions with other elements.

Magnesium (Mg) is an essential co-factor in the enzymatic pathways involved with energy, protein and lipid metabolism and the modulation of glucose transport through the cellular membrane [3, 4]. A reduction in the intracellular concentration of magnesium provokes a fall in the activity of tyrosine kinase in the insulin receptor [5, 6] and reduces the uptake of glucose by interfering with the translocation of the glucose transporter [GLUT 4]. Furthermore, Mg deficiency induces oxidative stress [7, 8]. A diet rich in Mg improves the homeostasis of glucose and insulin [9-13]. Moreover, it has been shown that abnormal magnesium homeostasis occurs in diabetes, frequently accompanied by hypomagnesaemia.

The fact that both Mg and V participate in glucose metabolism, intervening in the same processes, led us to consider that these two elements could be linked and might be acting jointly in the metabolism of carbohydrates [14]. Scibior et al. [15-18] studied the possible relationship between V and Mg in healthy rats. However, no data have been published on whether exposure to V might alter the metabolism of Mg in diabetic rats.

In a previous paper [19], it was reported that V treatment reduced the absorption, retention, serum level and femur content of Mg in control rats and that treatment with V of Mg-deficient rats corrected many of the alterations that had been generated by Mg deficiency. Thus, we considered it of interest to determine whether V treatment might affect the alterations in Mg metabolism associated with diabetes. Therefore, the aim of the present study was to examine changes in the metabolism of Mg, and related blood parameters, following treatment with bis(maltolato)oxovanadium (IV) of STZ-induced diabetic rats. The results obtained might help to clarify the role of V as an antidiabetic agent, as well as its biological activity.

**Methods and material**

**Animals and diets**

Male Wistar rats weighing 190-220 g (Charles River Laboratories, L’Arbresle, France) were randomly divided into four groups. **Control group:** nine rats were fed the semi-synthetic diet AIN93M. This diet provided 456.4 mg Mg and 60 \( \mu \)g V/kg. **Diabetic group:** eight rats were fed the semi-synthetic diet AIN93M. **Diabetic group treated with 1 mg V/day:** 10 rats were fed the semi-synthetic diet AIN93M. In addition, the rats in this group received 6.22 mg BMOV/day in their drinking water, which supplied 1 mg V/day. **Diabetic group treated with 3 mg V/day:** 10 rats were fed the semi-synthetic diet AIN-93M. In addition, the rats in this group received 18.66 mg BMOV/day in their drinking water, which supplied 3 mg V/day.

In all cases, diabetes was induced in the rats by injection of streptozotocin at a dose of 60 mg/kg b.wt. To confirm diabetes, on day seven, blood samples were obtained from the tail and glucose concentrations were analysed. Animals with levels greater than 13.8 mmol/L were considered to be diabetic.

During the experimental period, the BMOV solution was prepared daily, and the weight gain and intake of food and water were monitored. Every seven days, the glucose level in peripheral blood was analysed. On day 35, the rats were anaesthetised with a solution of pentobarbital (0.5 g/100 mL, Sigma-Aldrich, St Louis, MO, USA), and exsanguinated by cannulating the posterior aorta. Blood was collected and centrifuged (Beckman, Fullerton, CA, USA) at 3,000 rpm for 15 min to separate the serum. The liver, gastrocnemius muscle, kidney, liver, spleen, heart and femur were removed, weighed, placed in preweighed polyethylene vials, and stored at -80°C. Over the last seven days of the experimental period, the faeces and urine were collected every 24 h and stored at -80°C in polyethylene bottles for subsequent analysis.

From day 0 of the experiment, all animals were housed in individual metabolic cages designed for the separate collection of faeces and urine. The cages were located in a well-ventilated, temperature-controlled room (21 ± 2°C) with relative humidity ranging from 40 to 60%, and a light:dark period of 12 h.
The following biological indices were calculated: absorbed as [I-F], absorption (%), as 
\[\frac{(I-F)}{I} \times 100\], retained, as [I-(F+U)], and (\%R/I) as 
\[\frac{[I-(F+U)]}{I} \times 100\], where I = intake, F = faecal excretion, and U = urinary excretion.

All experiments were undertaken according to Directional Guides Related to Animal Housing and Care (European Community Council, 1986), and all procedures were approved by the Animal Experimentation Ethics Committee of the University of Granada.

**Analytical methods**

Determination of V and Mg in the diet, serum and tissues was performed by ICP-MS (Agilent 7500, Tokyo, Japan). All of the materials used in the analysis were previously cleaned with super-pure nitric acid and ultra-pure water (18.2 Ω) obtained using a Milli Q system. Samples were prepared by digestion with nitric acid and hydrogen peroxide (super-pure quality, Merck), in a microwave digester (Milestone, Sorisole, Italy). When the sample had been digested, the extract was collected and made up to a final volume of 10 mL for subsequent analysis.

Calibration curves were prepared following the Ga addition technique as an internal standard, using stock solutions of 1,000 mg/L of each element (Merck). The total metal content (V and Mg) in the tissues was analysed using ICP-MS techniques, and the accuracy of the method was evaluated by analysis of suitable, certified, reference materials, Seronorm (Billingstad, Norway) and NIST 8414, (Gaithersburg, MD 20899) and by recovery studies in samples of organs enriched with multi-element standards. The %CV obtained for Mg was 1.9% and that for V 5.6%. For each element we used the mean of five separate determinations of this reference material.

Glycaemia levels were determined using the sensor ACCU-CHEK AVIVA (Roche-Mannheim, Germany). Serum levels of insulin were determined using the SPI BIO (Montigny le Bretonneux, France) enzyme immune assay technique. Albumin, uric acid, urea, total cholesterol, LDL cholesterol, triglycerides, aspartate aminotransferase and alkaline phosphatase were determined using a BS-200 Chemistry Analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Hamburg, Germany).

**Statistical analysis**

Descriptive statistical parameters (means and standard deviations) were obtained for each of the variables studied. Taking into account the difficulty of determining whether the data follow a normal distribution, because the sample size (eight values per group) did not have enough power to test the normality of the variable, the Mann Whitney U test for two independent samples and the Kruskal-Wallis test for multiple independent samples were used in the analysis. For the bivariate analysis, Spearman’s coefficient of correlation was calculated. All analyses were performed using the Statistical Package for Social Science 15.0 (SPSS, Chicago, IL, USA).

**Results**

As a consequence of gastrointestinal disorders, two rats were removed from each V-treated group. Table 1 shows the digestive and metabolic utilisation of Mg during the experimental period. Intake was higher in the diabetic and the diabetic group treated with 1 mg V/day than in the control group. However, the intake by the diabetic group treated with 3 mg V/day was similar to that of the control group and lower than that of the other diabetic groups. A similar pattern was observed with respect to faecal excretion. Urinary excretion was higher in the diabetic groups than in the control group. However, in the diabetic group treated with 3 mg V/day, urinary excretion was lower than in the other diabetic groups. The net values of absorbed Mg were higher in the diabetic group and in the diabetic group treated with 1 mg V/day than in the control group. Nevertheless, in the diabetic group treated with 3 mg V/day, the absorbed value was lower than in the other diabetic groups.

Retention values were lower in the diabetic group and in the treated diabetic groups than in the control group. When expressed as a percentage (% absorption and %R/I), no significant changes were observed in % absorption, but the percentage of retention was lower in the diabetic and diabetic treated groups than in the control group.

Table 2 shows serum V and Mg on day 35 in the experimental groups. The streptozotocin-induced diabetic rats showed a significant increase in serum V and a decrease in Mg. The rats treated with 1 mg V/day did not present any changes in
Table 1. Digestive and metabolic utilisation of magnesium on days 28-35 of the study, for control rats (C), diabetic streptozotocin rats (DM), diabetic streptozotocin rats treated with 1 mg V/day (DM-1 mg V/day), and diabetic streptozotocin rats treated with 3 mg V/day (DM-3 mg V/day).

<table>
<thead>
<tr>
<th></th>
<th>C (n = 9)</th>
<th>DM (n = 8)</th>
<th>DM-1 mg V/d (n = 8)</th>
<th>DM-3 mg V/d (n = 8)</th>
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<tr>
<td></td>
<td>Mean  SD</td>
<td>Mean  SD</td>
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<tr>
<td>Intake Mg (mg/day)</td>
<td>6.8  0.9</td>
<td>15.1  1.1</td>
<td>12.3  0.9</td>
<td>6.3  0.5</td>
</tr>
<tr>
<td>Faecal Mg (mg/day)</td>
<td>3.5  0.6</td>
<td>8.6  0.8</td>
<td>6.6  0.8</td>
<td>3.3  0.7</td>
</tr>
<tr>
<td>Urinary Mg (mg/day)</td>
<td>0.4  0.2</td>
<td>4.8  1.2</td>
<td>4.0  0.8</td>
<td>1.3  0.3</td>
</tr>
<tr>
<td>Absorbed Mg (mg/day)</td>
<td>3.3  0.9</td>
<td>6.5  1.1</td>
<td>5.7  1.3</td>
<td>3.0  0.6</td>
</tr>
<tr>
<td>Absorbed Mg (%)</td>
<td>48  9</td>
<td>43  5</td>
<td>46  8</td>
<td>48  9</td>
</tr>
<tr>
<td>Retained Mg (mg/day)</td>
<td>2.9  0.9</td>
<td>1.7  0.8</td>
<td>1.7  0.8</td>
<td>1.7  0.6</td>
</tr>
<tr>
<td>Retained Mg (%)</td>
<td>42  9</td>
<td>11  5</td>
<td>14  6</td>
<td>27  9</td>
</tr>
</tbody>
</table>

Values shown are means ±SD standard deviation; *Mean value was significantly different from that of the C group. †Mean value was significantly different from that of the DM-1mgV/d group. P<0.05. NS non-significant

Absorbed as [I-F]; absorption (%), as [(I-F)/I] x 100; retained, as [I-(F+U)]; and retained (%) as [I-(F+U)]/I x100, where I = intake, F = faecal excretion, and U = urinary excretion.

Table 2. Serum vanadium and magnesium on day 35 of the study, for control rats (C), diabetic streptozotocin rats (DM), diabetic streptozotocin rats treated with 1 mg V/day (DM-1mgV/d), and diabetic streptozotocin rats treated with 3 mg V/day (DM-3mgV/d).

<table>
<thead>
<tr>
<th></th>
<th>C (n = 9)</th>
<th>DM (n = 8)</th>
<th>DM-1mgV/d (n = 8)</th>
<th>DM-3mgV/d (n = 8)</th>
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<tbody>
<tr>
<td></td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>Mean  SD</td>
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<tr>
<td>Serum V (µmol/L)</td>
<td>0.045  0.004</td>
<td>0.12  0.009*</td>
<td>0.7*  0.62*</td>
<td>15.0  1.5*†</td>
</tr>
<tr>
<td>Serum Mg (mmol/L)</td>
<td>0.86  0.03</td>
<td>0.71  0.07*</td>
<td>0.62  0.07*</td>
<td>0.50  0.06*†</td>
</tr>
</tbody>
</table>

Values shown are means ±SD standard deviation; *Mean value was significantly different from that of the C group. †Mean value was significantly different from that of the DM-1mgV/d group. P<0.05.

serum Mg compared with the diabetic group. The group treated with 3mgV/day presented lower levels of serum Mg than the control and diabetic groups.

Figure 1 shows glycaemia and serum insulin levels on day 35 in the diabetic groups. The rats treated with 1 mg V/day presented increased glycaemia, but no changes in serum insulin compared with the diabetic group. The treatment with 3 mg V/day maintained glycaemia at levels similar to those found in the control group and presented lower levels of serum insulin than in the control group.

Table 3 shows the Mg content in the muscle, kidney, liver, spleen, heart and femur on day 35. Mg content decreased in the femur in the diabetic groups, in comparison with the control group. The diabetic group treated with 1 mg V/day presented a lower content of Mg in the spleen and femur than the control and diabetic groups. The diabetic group treated with 3 mg V/day presented a lower content of Mg in the kidney than the other diabetic groups, and a lower Mg content in the liver, spleen and femur than the control group. Table 4 shows the changes in serum albumin, uric acid, urea, total cholesterol, LDL cholesterol, triglycerides, aspartate aminotransferase and alkaline phosphatase, caused by the diabetes and the V treatment.

The bivariate study revealed the existence of a significant relationship, among which the following are particularly important: fasting glycaemia correlated positively with uric acid (r = 0.54,
Discussion

Vanadium is a trace element associated with the regulation of glucose, improving its transport and metabolism and increasing the sensitivity of the insulin receptor [1]. Although some V compounds are currently undergoing human clinical trials [1], many aspects remain to be determined, such as its interactions with other elements. The present study was designed to obtain information on possible changes in the metabolism and distribution of Mg, and any associated alterations in biochemical parameters, following exposure to V. The results obtained reveal the existence or otherwise of interactions between these two elements in diabetic rats and could help to clarify the role of V as an anti-diabetic agent, as well as its toxicity.

The reasons for the specific doses used, and observations of toxicity problems observed in the animals thus treated, have been described in previous publications [19-21].

In the untreated diabetic rats, hyperphagia associated with diabetes led to increased intake of food and therefore of Mg. The absence of significant changes in the % absorption of Mg suggests that the increase in the net absorption of the cation in these rats is a consequence of the increased intake (table 1). Despite the increased net absorption, the sharp increase in urinary losses of Mg as a result of the polyuria caused by the altered endocrine state led to Mg retained in this group being less than in the control group (table 1), and also produced a decrease in plasma levels (table 2) [22].

The dose of 1 mg V/d did not produce any metabolic correction (figure 1), and so the behaviour of this group was similar to that of the group of untreated diabetic rats. However, serum Mg levels presented a downward trend. Previous studies performed by our research group showed that the treatment of healthy rats with 1 mg V/day also causes a decrease in serum Mg [19]. It is known that V stimulates Mg uptake in erythrocytes [14], this fact could have contributed to the hypomagnesaemia.

An earlier study [20] showed that treatment with 3 mg V/day produces a hypoglycaemic effect, normalising fasting blood glucose levels (figure 1) and food intake (table 1). Reduced intake causes a decrease in net Mg absorption, but does not modify the % absorption of Mg, which suggests that, at this dose, the changes observed in absorbed Mg
Table 3. Magnesium content in muscle, kidney, liver, spleen, heart and femur (mg/kg dry tissue) on day 35 for control rats (C), diabetic streptozotocin rats (DM), diabetic streptozotocin rats treated with 1 mg V/day (DM-1mgV/d) and diabetic streptozotocin rats treated with 3 mg V/day (DM-3mgV/d).

<table>
<thead>
<tr>
<th></th>
<th>C (n = 9)</th>
<th>DM (n = 8)</th>
<th>DM-1mgV/d (n = 8)</th>
<th>DM-3mgV/d (n = 8)</th>
<th>P&lt; K-W test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrocnemius muscle</td>
<td>900 45</td>
<td>930 46</td>
<td>983 405</td>
<td>874 78</td>
<td>NS</td>
</tr>
<tr>
<td>Kidney</td>
<td>860 112</td>
<td>854 37</td>
<td>879 119</td>
<td>791 44†</td>
<td>NS</td>
</tr>
<tr>
<td>Liver</td>
<td>922 123</td>
<td>849 130</td>
<td>817 111</td>
<td>787 35*</td>
<td>NS</td>
</tr>
<tr>
<td>Spleen</td>
<td>656 38</td>
<td>647 56</td>
<td>596 46*</td>
<td>600 71*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Heart</td>
<td>713 67</td>
<td>719 106</td>
<td>706 51</td>
<td>676 79</td>
<td>NS</td>
</tr>
<tr>
<td>Femur</td>
<td>2,610 107</td>
<td>2,424 145</td>
<td>1,869 367‡</td>
<td>1,972 229‡</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values shown are means ±SD standard deviation.

*Mean value was significantly different from that of the C group.
†Mean value was significantly different from that of the DM group.
‡Mean value was significantly different from that of the DM-1mgV/d group.

Table 4. Blood parameters on day 35 for control rats (C), diabetic streptozotocin rats (DM), diabetic streptozotocin rats treated with 1 mg V/day (DM-1mgV/d), and diabetic streptozotocin rats treated with 3 mg V/day (DM-3mgV/d).

<table>
<thead>
<tr>
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<th>DM (n = 8)</th>
<th>DM-1mgV/d (n = 8)</th>
<th>DM-3mgV/d (n = 8)</th>
<th>P&lt; K-W test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/L)</td>
<td>38 3</td>
<td>37 8</td>
<td>42 6</td>
<td>44 8</td>
<td>NS</td>
</tr>
<tr>
<td>Uric acid (µmol/L)</td>
<td>77 18</td>
<td>125 24*</td>
<td>262 71*</td>
<td>137 36*†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>13 2</td>
<td>35 5*</td>
<td>36 2</td>
<td>26 3*†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>1.9 0.2 2.4 0.4*</td>
<td>3.8 0.4*†</td>
<td>2.5 0.1*†</td>
<td>2.5 0.1*†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>0.9 0.2 1.1 0.2*</td>
<td>2.2 0.2*†</td>
<td>1.6 0.1*†</td>
<td>1.6 0.1*†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.8 0.2 1.2 0.07*</td>
<td>1.3 0.1*</td>
<td>1.1 0.08*†</td>
<td>1.1 0.08*†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/L)</td>
<td>98 10 137 15*</td>
<td>88 11†</td>
<td>72 8*†</td>
<td>72 8*†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>91 11 189 17*</td>
<td>172 21*</td>
<td>74 9*†</td>
<td>74 9*†</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values shown are means ±SD standard deviation.

*Mean value was significantly different from that of the C group.
†Mean value was significantly different from that of the DM group.
‡Mean value was significantly different from that of the DM-1mgV/d group.

P<0.05. NS: non-significant

are due to changes in intake, and that there are no interactions between the two elements in the digestive system.

The Mg retained in the diabetic group treated with 3 mg V/day was similar to that found in the other diabetic groups (table 1). However, serum Mg levels (table 1) were much lower than in the other groups. This fact could be explained on the basis that treatment of diabetic rats at a dose of 3 mg Vd produces higher renal losses of Mg than those found in the control group. The normalisation of the glycaemia (and consequently of the osmotic diuresis) found in diabetes could lead to an assumption that the renal losses of Mg would improve. However, our results indicate that glycaemic control with V does not correct the renal losses of Mg observed in diabetic rats that worsen the Mg status. It has been found that V treatment of diabetic rats reduces renal Na⁺, K⁺-ATPase activity, but this does not occur in non-diabetic rats [23]. The decrease in the activity of renal Na⁺, K⁺-ATPase in diabetic rats may explain the renal losses of Mg. However, we also observed increased renal losses in both diabetic and non-diabetic rats treated with V [19]. In our opinion, in addition to the possible inhibition of renal Na⁺,
K⁺-ATPase activity, other renal processes could have been altered; for example the V may block the transport of Mg and/or alter the expression of Mg transporter proteins in the kidney.

The diabetic rats presented lower levels of Mg in the femur with respect to the control group, which could be the result of a mobilisation of the cation in order to prevent a further decline in serum levels and to maintain homeostasis of the cation [24]. This tissue is the largest reservoir of Mg in the body [25].

In the 1 mg V/day group, the mobilisation of Mg affected both the bone and the spleen. However, in the 3 mg V/day group, Mg mobilisation was observed in all of the tissues studied, although in the case of the heart, the difference was not statistically significant (table 3). In the latter group, the hyperphagia was corrected and intake remained similar to that of the control group, although renal excretion was higher. This situation, coupled with increased Mg uptake by erythrocytes, may have been responsible for the increased hypomagnesaemia. In our opinion, the cause of the greater mobilisation of Mg from some tissues toward the extracellular space was the need to maintain ion homeostasis.

To determine whether the hypomagnesaemia caused by the diabetes and aggravated by the V treatment (table 2) affected the metabolic status of the rats, we examined certain biochemical parameters related to the metabolism of Mg (table 4) [26]. The results obtained show that diabetes causes an increase in the levels of circulating uric acid and urea, which may be due to an increased catabolism of amino acids [27] and to the existence of kidney problems [28]. Furthermore, it has been reported that even when glycaemia is controlled with insulin, levels of urea remain significantly increased [27].

Treatment with 1 mg V/day provoked a sharp rise in the levels of both metabolites, while a dose of 3 mg V/day significantly reduced both values. However, in the latter group, the uric acid levels were similar to those found in untreated diabetic rats but higher than those of the control rats; urea levels remained above those of the control rats. Increased levels of plasma urea in diabetic rats exposed to V have also been observed when other compounds of V are used [29, 30].

Furthermore, it is known that Mg deficiency affects the metabolism of proteins and nucleic acids [26, 31, 32]. Our results indicate that diabetes produces hypomagnesaemia, which worsens with V treatment, in a dose-related fashion. In addition, serum Mg levels correlated negatively with those of uric acid and urea (see results). This suggests that hypomagnesaemia may be involved in the above-mentioned metabolic disorders.

The mobilisation of fatty acids as a result of diabetes is known to produce alterations in lipid metabolism. It is also known that Mg deficiency alters the metabolism of lipoproteins by reducing the activity of lecithin-cholesterol acyltransferase and of lipoprotein lipase [33-35].

In the present study, diabetes was also found to increase total-cholesterol, LDL-cholesterol and triglycerides. The treatment with 1 mg V/day increased total-cholesterol and LDL-cholesterol, in comparison with untreated diabetic rats, whereas a dose of 3 mg V/day produced a downward trend in these parameters in comparison with a dose of 1 mg V/day, approaching those found in the untreated diabetic rats. In relation to the effect of V treatment on the lipid profile in diabetic rats, the literature is contradictory. Although some authors have observed increases in cholesterol [36], others have not [37], while various authors [30, 36, 38, 39] have found that in treated diabetic rats, the levels of circulating cholesterol and triglycerides tend to decrease, but in many cases the reduction is only partial. In general, the effects obtained vary greatly depending on the compound, dose, and duration of treatment. Comparing our results with those obtained with V doses close to the highest one used in our study (3 mg V/day), some authors, using a slightly lower dose, reported no changes in levels of cholesterol and triglycerides [37], and others, at a slightly higher dose, only observed a small decrease in cholesterol levels, compared to untreated diabetic rats [30]. In neither of the cases discussed were data on Mg presented.

The fact that at a dose of 3 mg V/day, levels of urea, uric acid, total-cholesterol and triglycerides tended to fall in comparison with the dose of 1 mg V/day, approaching values found in the untreated diabetic rats – despite the fact that hypomagnesaemia found in the group treated with the dose of 3 mg V/day remained higher than in the group treated at the dose of 1 mg V/day – led us to consider that the metabolic improvement found in the diabetic group treated at the highest dose tested could be related to a direct effect of V on the lipid and protein metabolism, acting not only as a hypoglycaemic agent, but also as an insulin-mimetic agent [1, 2]. Hypomagnesaemia would tend to
aggravate the metabolic alterations caused by diabetes, while the improvement in lipid and protein metabolism derived from the insulin-mimetic effect of vanadium would favour its normalisation. This would explain the fact that the higher dose reduced these values, even though the levels in the control rats were not reached.

Alkaline phosphatase and aspartate aminotransferase exhibit similar behaviour patterns; diabetes increases their activity, while V treatment tends to decrease it, with a dose of 3 mg V/day causing the activity of both enzymes to fall below that of the control rats. There is no current consensus as to how V affects the activity of these enzymes. Some authors have described a partial normalisation of their activity after the exposure of diabetic rats to V [30], but others have found no such effect, or have obtained indeterminate results [29, 40]. Alkaline phosphatase is known to be an Mg-dependent enzyme [26] that is necessary for the uptake of pyridoxal-5-phosphate (vitamin B₆) by the tissues, and this vitamin is a coenzyme of aspartate aminotransferase. Furthermore, Mg deficiency is associated with decreased alkaline phosphatase activity and a deficit of vitamin B₆ [41]. In our opinion, hypomagnesaemia may also account for the decrease in alkaline phosphatase activity and thus in aspartate aminotransferase activity. The positive correlations between aspartate aminotransferase activity, serum Mg and alkaline phosphatase (see results), support this opinion.

In conclusion, under our experimental conditions, treatment with 3 mg V/day, as BMOV, to diabetic rats normalised glycaemia, but increased the renal losses of Mg, that induced tissue depletion of Mg. This effect could have partially contributed to the fact that the highest dose of V tested, failed to normalise the lipid and protein metabolism biomarkers studied. However, further studies of Mg dietary supplementation in diabetic rats are needed to determine better the effects arising from these interactions.

Disclosure

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