Bioavailability, tissue distribution and hypoglycaemic effect of vanadium in magnesium-deficient rats

Cristina Sánchez¹, Miguel Torres¹, María C. Bermúdez-Peña³, Pilar Aranda¹, María Montes-Bayón², Alfredo Sanz-Medel², Juan Llopis¹

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

Correspondence: 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 <jllopis@ugr.es>

Abstract. Vanadium is an element whose role as a micronutrient and hypoglycaemic drug has yet to be fully clarified. The present study was undertaken to investigate the bioavailability and tissue distribution of vanadium and its interactions with magnesium in healthy and in magnesium-deficient rats, in order to determine its role as a micronutrient and antidiabetic agent. Four groups were used: control (456.4 mg magnesium and 0.06 mg vanadium/kg food); control treated with 1 mg vanadium/day; magnesium-deficient (164.4 mg magnesium/kg food and 0.06 mg vanadium/kg food); and magnesium-deficient treated with 1 mg vanadium/day. The vanadium was supplied in the drinking water as bis(maltolato)oxovanadium (IV). The experiment had a duration of five weeks. We measured vanadium and magnesium in excreta, serum, skeletal muscle, kidney, liver, adipose tissue and femur. Fasting glucose, insulin and total antioxidant status (TAS) in serum were studied. The vanadium treatment applied to the control rats reduced the absorption, retention, serum level and femur content of magnesium. Magnesium deficiency increased the retention and serum level of vanadium, the content of vanadium in the kidney, liver and femur (organs where magnesium had been depleted), serum glycaemia and insulin, and reduced TAS. V treatment given to magnesium-deficient rats corrected magnesium content in muscle, kidney and liver and levels of serum glucose, insulin and TAS. In conclusion, our results show interactions between magnesium and vanadium in the digestive and renal systems. Treatment with vanadium to magnesium-deficient rats corrected many of the alterations that had been generated by the magnesium deficiency.

Key words: vanadium, magnesium, metabolism interactions, glycaemia, insulin, rat

Magnesium plays a structural and regulatory role in the organism. Among its functions one of the most important is to act as the ion that activates a large number of enzyme systems, making magnesium essential for the metabolism of many nutrients.

From a physiological standpoint, the functions of magnesium are well known and have been amply described in many publications. In another area of research, most epidemiological studies have reported that in western countries, magnesium intake is below the recommended...
Vanadium bioavailability in magnesium deficient rats

allowances in considerable percentages of the population. According to published data, the amounts of magnesium consumed are below 80% of the recommended intake in more than 20% of the population. In addition, hypomagnesaemia has been found in substantial proportions of the adult population in Europe [1, 2].

The clinical manifestations of magnesium deficiency are difficult to define because depletion of this cation is associated with considerable abnormalities in the metabolism of many elements and enzymes. However, if suboptimal intake is prolonged it can facilitate or cause the appearance of symptoms currently attributed to other causes, or whose causes are unknown. Some examples of this situation are the relationships between magnesium deficiency and cardiovascular, renal, gastrointestinal, neurological, muscular and bone disorders [1]. Furthermore, there is a well known direct relation between nutritional magnesium deficit, resistance to insulin, metabolic and glucose transport disorders and the risk of developing type II diabetes [3-10]. This mechanism is based on the fact that the reduction in the intracellular concentrations of magnesium raises the intracellular levels of Ca [11-13], which brings about a fall in the activity of tyrosine kinase in the insulin receptor [14]. In addition, the intracellular deficit of magnesium reduces the uptake of glucose by interfering with the translocation of the glucose transporter [GLUT 4]. Intracellular magnesium depletion also reduces the intracellular utilisation of glucose (magnesium is a cofactor of the enzymes involved in glucose oxidation) thus provoking peripheral resistance to insulin [15]. Furthermore, it has been observed that magnesium deficiency induces oxidative stress. These circumstances facilitate the development of type II diabetes [12, 13, 16-18].

Vanadium is a widely distributed element on earth, but its role as a micronutrient in humans is not yet fully established. In plasma, it is bound to plasma proteins, primarily transferrin. It is distributed primarily in the bone, kidney and liver. However, the mechanism of absorption, transport and disposal of vanadium is not well established.

Some complexes of vanadium (IV) have been shown to possess hypoglycaemic or insulin mimetic properties, in type II diabetes patients, stimulating the autophosphorylation of the insulin receptors, increasing the activity of tyrosine kinase and favouring the translocation of the transported GLUT 4 [19-21]. Vanadium also alleviates oxidative stress, by improving the metabolism of glucose, which reduces the production of free radicals resulting from disorders in glucose metabolism [20], and thus prevents the development of the diabetes process.

The fact that both magnesium and vanadium participate in glucose metabolism, intervening in the same processes, led us to consider that these two elements could be related and be acting jointly in the metabolism of carbohydrates. Therefore, they may present metabolic interactions.

The aim of this study was to examine the digestive and metabolic utilisation of vanadium and its interactions with magnesium in control and magnesium-deficient rats. This paper studies the hypoglycaemic effects of vanadium compounds, considering the magnesium-deficient rat as a model of prediabetes. Vanadium was supplied as bis(maltolato)oxovanadium (IV) (BMOV), a vanadium complex that is 2 to 3 times more effective than inorganic vanadium as a glucose-lowering agent [19, 20]. The results obtained clarify its role as a micronutrient and anti-diabetic agent.

Materials and methods

Animals and diets

Male Wistar rats weighing 190-220 g (Charles River Laboratories, Barcelona, Spain) were randomly divided into 4 groups of 8 rats in which the amount of dietary magnesium and vanadium was varied.

Control group: 8 rats fed with the semisynthetic diet AIN-93 [22]. This diet provided 456.4 mg magnesium and 0.06 mg vanadium/kg food.

Control treated with vanadium group: 10 rats fed with the AIN-93 diet. In addition, the rats in this group received in their drinking water 6.22 mg bis(maltolato)oxovanadium (IV)/day, which supplied 1mg vanadium/day.

Magnesium deficient group: 8 rats fed with the diet AIN-93 deficient in magnesium. The diet contained AIN-93 mineral mix without magnesium oxide. This diet supplied 164.4 mg magnesium/kg food.

Magnesium deficient treated with vanadium group: 10 rats fed with the diet AIN-93 deficient in magnesium. The diet contained AIN-93 mineral mix without magnesium oxide. This diet supplied 164.4 mg magnesium/kg food. In addition,
the rats in this group received in their drinking water 6.22 mg bis(maltolato)oxovanadium (IV)/day, which supplied 1 mg vanadium/day.

In all cases, the bis(maltolato)oxovanadium (IV) solution was prepared daily. During the experimental period, the weight gain and the intake of food and water were monitored. Every seven days, the glucose level in peripheral blood was analysed. On day 35 the rats were anaesthetized 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 Coulter, California, USA) at 3,000 rpm for 15 minutes to separate serum. The liver, gastrocnemius muscle, kidney, perirenal adipose tissue and femur were also removed, weighed, placed in preweighed polyethylene vials, and stored at -80°C. During the last 7 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.

All animals were housed from day 0 of the experiment 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°C ± 2) 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 [(I-F)/I] × 100, Retained, as [I-(F+U)], and (%R/I) as [(I-(F+U)/I) × 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 Council Community, 1986) and all procedures were approved by the Animal Experimentation Ethics Committee of the University of Granada.

Analytical methods

Determination of magnesium and vanadium in the diet, serum and tissues was performed by ICP-MS (Agilent 7500, Agilent Technologies, Tokyo, Japan). All the materials used in the analysis were previously cleaned with super-pure nitric acid, and ultrapure 18 MΩcm distilled de-ionized water was obtained by means of a Milli-Q system (Millipore, Bedford, MA, USA).

Samples were prepared by attack 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 dilution of 1/10 (w/v) 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, Darmstadt, Germany).

The total metal content (magnesium and vanadium) in the tissues was analysed using ICP-MS techniques [23], and the accuracy of the method was evaluated by analysis of suitable certified reference materials Seronorm, (Billingstad, Norway) and NIST 8414, (Gaithersburg, MD 20899, USA) and by recovery studies in samples of organs enriched with multi-element standards.

Glycaemia levels were determined using the sensor ACCU-CHEK AVIVA (Roche-Mannheim, Germany). Plasma levels of insulin were determined using the SPI BIO (Montigny le Bretonneux, France) enzyme immuno assay technique. The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using the formula [fasting glucose (μIU/mL) × fasting glucose (mg/dL)] / 405.

Total antioxidant status (TAS) was determined using a Randox® assay kit (Randox Laboratories Ltd. Crumlin, Co. Antrim, UK). The reagent measures inhibition of the absorbance of the radical cation of 2,2 azinobis (3-ethylbenzothiazolin 6-sulfonate) (ABTS), which has a long-wave length absorption spectrum showing maxima at 660 nm. The ABTS radical cation is formed by the interaction of ABTS (150 mmol) with the ferrylmyoglobin radical species generated by the activation of metmyoglobin (2.5 mmol) with H2O2 (75 mmol). Antioxidant compounds suppress the absorbance of the ABTS radical cation to a degree and on a time scale dependent on the antioxidant capacity. Decolourisation was used as the assay endpoint. This reaction requires the presence of myoglobin, acting as a peroxidase through the formation of the ferrylmyoglobin radical, to which ABTS donates an electron, forming ABTS+. When an aliquot of sample is added to the reaction mixture, there is a degree of decolourisation owing to the presence of plasma antioxidants that reverse the formation of the ABTS radical cation. The per cent loss of blue-green (blank-test measuring at 734
vanadium bioavailability in magnesium deficient rats

nm) was used as an index of plasma antioxidant status.

Statistical analysis

Descriptive statistical parameters (means and standard deviations) were obtained for each of the variables studied. The experimental data were analysed using the tests for nonparametric samples, using the Mann Whitney U test for independent samples. To discern the possible interactions between vanadium and magnesium treatment, two-way analysis of variance (two-way ANOVA) was used. For the bivariate analysis, Pearson’s coefficient of correlation was calculated. All the analyses were performed using Statistical Package for Social Science 15.0 (SPSS, Chicago, IL, USA). Differences were considered statistically significant at a probability level of 5%.

Results

Figure 1 shows the evolution of the mean weight of the rats in the experimental period and Figure 2 shows that of fasting glycaemia during the same period. The results obtained show that consumption of the magnesium-deficient diet significantly increased glycaemia. The vanadium treatment given to magnesium-deficient rats maintained glycaemia levels similar to those of the controls.

Table 1 shows serum magnesium, vanadium and insulin, the HOMA-IR index and TAS. The magnesium-deficient diet significantly increased levels of circulating insulin and HOMA-IR, while the treatment given to the deficient rats returned these levels to values similar to those of the controls. Consumption of the magnesium-deficient diet led to a fall in TAS. The vanadium treatment given to the deficient rats returned these levels to values similar to those presented by the control animals.

Table 2 shows the digestive and metabolic utilisation of magnesium and vanadium during the final weeks of the study (days 28-35), in the four experimental groups. Vanadium treatment to control rats produced a significant increase in the percentage of vanadium absorbed and retained, as well as a significant increase in the urinary excretion of magnesium, together with a decrease in magnesium retention.

Figure 1. Evolution of mean body weight in rats during the experimental period (mean±SD, n=8).

a Control vs vanadium treated; b control vs magnesium deficient; c control vs magnesium deficient treated with vanadium; d vanadium treated vs magnesium deficient; e vanadium treated vs magnesium deficient treated with vanadium; f magnesium deficient vs magnesium deficient treated with vanadium. P <0.05.
Consumption of the magnesium-deficient diet produced a significant reduction in urinary losses of vanadium and increased vanadium retention, together with higher serum vanadium levels (table 2).

Vanadium treatment in the rats given the magnesium-deficient diet increased the absorption and retention of vanadium. These increases were smaller than those found in the treated control rats.

Although no significant changes were observed in magnesium concentration in the adipose tissue following consumption of the magnesium-deficient diet, a downward tendency in these values was observed. On the other hand, there was a significant reduction in magnesium in the skeletal muscle, kidney, liver and femur. In addition, the magnesium-deficient diet slightly raised vanadium content in the muscle, liver and kidney (table 3).

Vanadium treatment produced a significant reduction in magnesium in the femur. The vanadium treatment given to the magnesium-deficient rats increased the content of magnesium in the kidney and liver, eliminating the differences produced by magnesium deficit with respect to the control rats. Nevertheless, there was no change in the level of magnesium content in the femur (table 3).

The bivariate study revealed the existence of a high number of significant relations, among which the following are particularly important. Ingested magnesium correlated inversely with glycaemia, serum insulin and HOMA ($r=-0.461$, $r=-0.507$ and $r=-0.468$ respectively; $p<0.01$ in every case). Ingested vanadium also correlated inversely with glycaemia, insulin and HOMA ($r=-0.463$, $r=-0.501$ and $r=-0.514$; $p<0.01$). TAS correlated positively with the intake of magnesium ($r=0.500$; $p<0.01$) and serum levels of vanadium ($r=0.458$; $p<0.01$) and negatively with glycaemia, serum insulin and HOMA ($r=-0.567$, $r=-0.568$ and $r=-0.677$ respectively; $p<0.01$ in every case).

### Discussion

The dose of vanadium used in this study (6.22 mg bis(maltolato)oxovanadium (IV)/day or ∼0.6 mg bis(maltolato)oxovanadium (IV)/mL, which supplied 1mg vanadium/day or ∼5mg vanadium/kg of diet).
Table 1. Fasting magnesium (Mg), vanadium (V) and insulin content in serum, HOMA-IR and Total Antioxidant Status on day 35, of control rats, treated with vanadium (1mg vanadium/day), magnesium deficient and magnesium deficient treated with vanadium (1mg vanadium/day) for 35 days.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vanadium treated</th>
<th>Magnesium deficient</th>
<th>Magnesium deficient treated with V</th>
<th>ANOVA P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Main effect of Mg</td>
</tr>
<tr>
<td>Serum Mg (mg/L)</td>
<td>21.00 ± 1.02</td>
<td>17.11 ± 2.01 a</td>
<td>8.42 ± 1.34 a,b</td>
<td>9.25 ± 2.64 a,b</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Serum V (µg/L)</td>
<td>2.35 ± 0.57</td>
<td>456.73 ± 52.87 a</td>
<td>3.03 ± 0.68 a,b</td>
<td>439.62 ± 141.96 a,c</td>
<td>NS</td>
</tr>
<tr>
<td>Serum Insulin (ng/mL)</td>
<td>2.05 ± 0.78</td>
<td>1.73±0.81</td>
<td>6.65 ± 3.72 a,b</td>
<td>1.82 ± 1.49 c</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>HOMA-IR index*</td>
<td>9.53 ± 4.36</td>
<td>7.88 ± 3.57</td>
<td>39.63 ± 22.04 a,b</td>
<td>7.69 ± 5.64 c</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Total Antioxidant Status (mmol/L)</td>
<td>1.29 ± 0.30</td>
<td>1.37 ± 0.15</td>
<td>0.77 ± 0.33 a,b</td>
<td>1.29 ± 0.13 c</td>
<td>p&lt;0.02</td>
</tr>
</tbody>
</table>

* Homeostatic model assessment for insulin resistance.
Values shown are means±SD, n=8 rats.

a Control vs vanadium treated, magnesium-deficient and magnesium-deficient treated;
b vanadium treated vs magnesium-deficient and magnesium-deficient treated;
c magnesium-deficient vs magnesium-deficient treated. P<0.05. NS no significant effect.
Table 2. Digestive and metabolic utilization of magnesium (Mg) and vanadium (V) on days 28-35 of study, for control rats, treated with vanadium (1mg vanadium/day), magnesium deficient and magnesium deficient treated with vanadium (1mg vanadium/day) for 35 days.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vanadium treated</th>
<th>Magnesium deficient</th>
<th>Magnesium deficient treated with vanadium</th>
<th>ANOVA P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Main effect of Mg</td>
</tr>
<tr>
<td><strong>Intake</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg (mg/day)</td>
<td>6.83±0.89</td>
<td>5.86±0.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.31±0.44&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>2.33±0.49&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>V (μg/day)</td>
<td>0.90±0.11</td>
<td>1038±208&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.84±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>989±127&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><strong>Faecal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg (mg/day)</td>
<td>3.53±0.93</td>
<td>3.44±1.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.80±0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.92±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>V (μg/day)</td>
<td>0.66±0.12</td>
<td>500±103&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.70±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>539±115&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td><strong>Urinary</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg (mg/day)</td>
<td>0.37±0.43</td>
<td>1.38±1.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.16±0.31&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.11±0.05&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>p&lt;0.02</td>
</tr>
<tr>
<td>V (μg/day)</td>
<td>0.17±0.04</td>
<td>67.9±28.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05±0.02&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>61.8±31.7&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td><strong>Absorbed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg (mg/day)</td>
<td>3.30±1.23</td>
<td>2.41±0.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.52±0.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.42±0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>V (μg/day)</td>
<td>0.23±0.11</td>
<td>538±93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.19±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>349±99.2&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><strong>Absorption</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg (%)</td>
<td>47.78±14.41</td>
<td>41.31±16.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.16±12.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.52±8.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>V (%)</td>
<td>25.70±12.31</td>
<td>51.91±9.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.93±14.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.3±8.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><strong>Retained</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg (mg/day)</td>
<td>2.93±1.64</td>
<td>1.02±1.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.36±0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.30±0.44&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>V (μg/day)</td>
<td>0.06±0.14</td>
<td>470±99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14±0.13&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>287±92.5&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><strong>% R/I&lt;sup&gt;1&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg (%)</td>
<td>42.90±13.2</td>
<td>17.40±14.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.84±11.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.79±7.22&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>V (%)</td>
<td>6.33±15.88</td>
<td>45.36±7.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.00±14.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.46±9.03&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

Values shown are means ± SD, n=8 rats. <sup>1</sup>% R/I, percentage of retention to intake.

<sup>a</sup> Control vs vanadium treated, magnesium-deficient and magnesium-deficient treated
<sup>b</sup> vanadium treated vs magnesium-deficient and magnesium-deficient treated
<sup>c</sup> magnesium-deficient vs magnesium-deficient treated. P<0.05. NS: no significant effect.
**Table 3.** Magnesium (Mg) and vanadium (V) content in muscle, kidney, liver, adipose tissue and femur (mg/kg dry tissue) on day 35, of control rats, treated with vanadium (1mg vanadium/day), magnesium deficient and magnesium deficient treated with vanadium (1mg vanadium/day) for 35 days.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vanadium treated</th>
<th>Magnesium deficient</th>
<th>Magnesium deficient treated with vanadium</th>
<th>ANOVA P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Main effect of Mg</td>
<td>Main effect of V</td>
<td>Interactive effect of Mg × V</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>899.86±44.87</td>
<td>901.01±72.11</td>
<td>808.48±99.64</td>
<td>879.73±37.10</td>
<td>p&lt;0.02 NS</td>
</tr>
<tr>
<td>V</td>
<td>0.014±0.008</td>
<td>1.068±0.156</td>
<td>0.015±0.002</td>
<td>0.923±0.286</td>
<td>NS p&lt;0.001 NS</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>859.59±111.72</td>
<td>867.82±106.83</td>
<td>749.66±52.95</td>
<td>768.70±37.94</td>
<td>p&lt;0.01 NS</td>
</tr>
<tr>
<td>V</td>
<td>0.12±0.023</td>
<td>28.11±5.97</td>
<td>0.168±0.050</td>
<td>25.51±4.49</td>
<td>NS p&lt;0.001 NS</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>922.07±123.47</td>
<td>900.34±141.96</td>
<td>794.11±76.42</td>
<td>869.80±129.59</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>V</td>
<td>0.043±0.010</td>
<td>8.77±2.50</td>
<td>0.061±0.014</td>
<td>8.81±1.63</td>
<td>NS NS p&lt;0.001 NS</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>19.67±5.31</td>
<td>17.16±7.08</td>
<td>14.72±4.27</td>
<td>16.58±7.18</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>V</td>
<td>0.007±±0.0039</td>
<td>0.239±0.058</td>
<td>0.0042±0.0019</td>
<td>0.251±0.096</td>
<td>NS p&lt;0.001 NS</td>
</tr>
<tr>
<td>Femur</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>2609.62±107.203</td>
<td>2130.16±312.68</td>
<td>1526.15±383.96</td>
<td>1478.53±366.62</td>
<td>p&lt;0.001 NS</td>
</tr>
<tr>
<td>V</td>
<td>0.034±0.013</td>
<td>15.78±4.96</td>
<td>0.079±0.016</td>
<td>19.52±6.68</td>
<td>NS p&lt;0.001 NS</td>
</tr>
</tbody>
</table>

Values shown are means±SD, n=8 rats.

- **a** Control vs vanadium treated, magnesium-deficient and magnesium-deficient treated.
- **b** vanadium treated vs magnesium-deficient and magnesium-deficient treated.
- **c** magnesium-deficient vs magnesium-deficient treated. P<0.05. **NS:** no significant effect.
were also observed by Scibior et al. [27] using ~9.5 mg vanadium/b.w./day in healthy Wistar rats. In our research we found that the growth curves for control and magnesium-deficient rats did not differ significantly. This finding has also been reported by other authors who used the AIN-93 diet [22] with similar magnesium content [30].

Treatment with 1 mg vanadium/day (as bis(maltolato)oxovanadium (IV)) to control rats produced a lower weight gain (figure 1). We also observed that the HOMA-IR correction in the group of rats given the magnesium-deficient diet, treated with vanadium, did not correct the weight gain curve. However, food consumption by all groups presented very similar levels (data not shown). On the other hand, we found no significant correlations between body weight changes and HOMA-IR (r=0.34; p>0.05), although there was a significant inverse correlation between body weight changes and vanadium intake (r=-0.44; p<0.01). These facts lead us to believe that vanadium treatment is the sole factor responsible for the low weight gain observed among these groups [31] and that, in our study, these weight losses were not related to the HOMA-IR normalisation found in the magnesium-deficient animals treated.

Epidemiological and clinical research suggests that low serum magnesium levels are associated with resistance to insulin and increase the risk of type II diabetes [3-10], and that oral magnesium supplementation improves insulin sensitivity and metabolic control in type II diabetic patients with decreased serum magnesium levels [32]. In our study, consumption of the magnesium-deficient diet produced hypomagnesaemia accompanied by a significant increase in glycaemia (figure 2) and in serum levels of insulin. In vitro and in vivo studies have shown that magnesium deficiency deteriorates insulin secretion and that magnesium supplementation prevents the development of diabetes in rats [33, 34]. The intracellular deficit of magnesium reduces the uptake of glucose by interfering with the translocation of the glucose transporter [GLUT 4]. Intracellular magnesium depletion also reduces the intracellular utilisation of glucose [15]. Furthermore, the reduction in the intracellular concentrations of magnesium promotes disorders in the activity of tyrosine kinase in the insulin receptor [14], these events being related to the development of insulin resistance. The increased HOMA-IR index in the magnesium-deficient rats confirmed the presence of increased resistance to insulin among the deficient animals (table 1). Moreover, the inverse correlations found between magnesium consumption and glucose, serum insulin and HOMA (see results) support the above findings.

It has recently been shown that vanadium is a trace element associated with the regulation of the glucose metabolism, improving its transport and metabolism and increasing the sensitivity of the insulin receptor [35]. Our results confirm these findings, showing that vanadium treatment given to rats consuming a magnesium-deficient diet maintains levels of glucose (figure 2), serum insulin and HOMA-IR (table 1) at values similar to those of the controls. In this case, too, the correlations found between V intake and levels of glucose, serum insulin and HOMA (see results) support the results given.

The results of the TAS study reveal the cumulative action of all the antioxidants present in the plasma. Thus, it is considered to be a reliable and sensitive biomarker of in vivo oxidative stress [36]. The reduction in TAS observed in the rats given the magnesium-deficient diet (table 1) is a consequence of the pro-oxidant capacity of magnesium deficiency [12, 13, 17, 37, 38]. It has been suggested that magnesium deficiency may provoke an increase in intracellular calcium [11-13, 37, 38]. Higher levels of cytosolic calcium would activate a whole set of factors (leukocyte and macrophage activation, the synthesis of inflammatory cytokines and acute phase proteins), giving rise to a pro-inflammatory effect [17, 38]. This inflammatory response would be the factor responsible for the increased...
production of free radicals and for the oxidative damage found with magnesium deficiency [12, 16, 36, 37]. A previous study reported a reduction in TAS among magnesium-deficient patients [39]. Vanadium treatment did not significantly modify TAS in the control rats. Other studies have failed to observe changes in plasma TAS following vanadium treatment [26, 27]. However, vanadium treatment of magnesium-deficient rats returned levels to values comparable to those of the controls (table 1). It has been reported that vanadium alleviates the oxidative stress process related to the appearance and development of diabetes, by improving the metabolism of glucose [20, 40]. The positive correlations of TAS with vanadium intake, serum vanadium and magnesium, and the negative correlations with glycaemia and insulin, support the above findings. Nevertheless, other authors have reported vanadium to have pro-oxidant effects [25, 41].

Magnesium absorption among both the control rats and the magnesium-deficient animals (table 2) presented values similar to those reported previously [42]. In magnesium-deficient groups, the percentage of absorption remained high, as expected under these experimental conditions. These changes reflected the adaptation of the digestive system under conditions of magnesium depletion, to compensate the insufficient dietary supply by increasing absorption. Treatment with vanadium did not modify the faecal excretion of magnesium (table 2). Therefore, at the digestive level, treatment with vanadium did not modify magnesium absorption.

Cation retention is the relationship between gastrointestinal and renal absorption and the process of excretion. The rats consuming a magnesium-deficient diet presented a cation retention value that was significantly lower than that of the control animals, as a consequence of the lower intake levels of this element. Expressing retained magnesium as a percentage of magnesium ingested, the groups given the magnesium-deficient diet presented higher values than the control animals, presenting a reaction pattern parallel to that of the absorption (table 2).

Vanadium absorption among the control animals presented values that were clearly higher than those described in previous publications [43, 44]. The higher absorption of vanadium found in our study could be related to the type of vanadium complex supplied and/or the different methods used to determine the presence of this element in the faeces.

Vanadium treatment produced an increased percentage of absorption of this element, which could be due in part to interactions with other elements in the digestive system. It has been reported that vanadium competes with divalent cations for binding sites [43]. Nevertheless, in our study the magnesium absorption in the animals treated with vanadium only presented a slight downward trend (table 2).

Although magnesium deficit increases the absorption of different elements [45-47], in the present study the magnesium-deficient diet was associated with slightly reduced vanadium absorption. Although these differences are not significant, the analysis of the variance for absorbed vanadium revealed the existence of interactions between magnesium and vanadium caused by treatments (table 2).

The vanadium treatment given to the control rats produced greater urinary losses of magnesium, causing reduced retention and lower magnesium retention. These losses account for the falls in serum levels of magnesium in this group (table 2). Moreover, it is known that vanadium stimulates magnesium uptake in erythrocytes [48]. The vanadium treatment given to magnesium-deficient rats did not affect urinary losses or magnesium retention, these values remaining similar to those observed in the non-treated deficient rats (table 2). However, consumption of the magnesium-deficient diet reduced urinary losses of vanadium and increased its retention, which explains the increase in serum levels of vanadium in the non-treated magnesium-deficient rats (table 1). In previous studies, our group found that the nutritional deficit of magnesium reduced the renal excretion of various elements [11, 45, 46].

These results show the existence of interactions in the kidney between magnesium and vanadium. The analysis of the variance with respect to retained vanadium confirmed the existence of significant interactions caused by the treatments (table 2).

The low level of magnesium retention observed in the control rats treated with vanadium is reflected in the lower levels of serum magnesium (table 1) and in the lower magnesium content in the femur (table 3), while in the other tissues studied no significant decreases were observed. The bone tissue constitutes the highest corporal
reserve of magnesium, and as a consequence of the reduced magnesium retention, this cation was mobilised in order to maintain its homeostasis [42, 49].

The intake of a magnesium-deficient diet led to a fall in the magnesium content in the skeletal muscle, kidney, liver and femur [42, 49], and an increase in the vanadium content in the same tissues (table 3). This raised level of vanadium deposits is a consequence of the higher retention and circulating levels of vanadium caused by a magnesium-deficient diet, as remarked above. It is interesting to observe that vanadium deposits were produced in the organs where magnesium had been depleted, which indicates the existence of possible interactions between these elements in the tissues in question. Moreover, in the case of the muscle and liver, the incorporation of vanadium could help alleviate the alterations to carbohydrate metabolism generated by the magnesium deficit.

The vanadium treatment given to control and magnesium-deficient rats produced an accumulation of vanadium in all the tissues studied. In the tissues of the magnesium-deficient rats treated with vanadium, magnesium depletion was only observed in the femur, with respect to the control group, which would indicate that the treatment prevented, at least in part, the mobilisation of magnesium from the muscle, kidney and liver tissues. The mechanism involved in this process could reflect the fact of vanadium increasing cellular magnesium uptake [47].

In summary, the vanadium treatment given to control rats reduced the retention of magnesium by the organism, producing a reduction in magnesium content in serum and in the femur, while the other parameters remained unchanged. Magnesium deficiency increased vanadium retention and vanadium deposits in the kidney, liver and femur. In addition, in the magnesium deficient group there was increased resistance to insulin, and a reduction in TAS. The vanadium treatment given to magnesium-deficient rats corrected many of the alterations that had been generated by the magnesium deficiency.

Therefore, we conclude that there are interactions between magnesium and vanadium in the digestive and renal systems. The increased hepatic deposits of vanadium observed in the magnesium-deficient rats could help alleviate the resistance to insulin generated by the magnesium deficit. Nevertheless, further studies are needed, assaying lower doses of vanadium in order to determine optimum levels for pharmacological use, reducing or avoiding toxic effects.

Disclosure

We are grateful for support received from the Consejería de Innovacion, Ciencia y Empresa, Andalusian Regional Government (Project P06-CTS-01435).

None of the authors has any conflict of interest to disclose.

References

9. Simental-Mendia LE, Rodríguez-Morán M, Guerrero-Romero F. Failure of β-cell function


20. Thompson KH, Orvig C. Vanadium in diabetes: 100 years from Phase 0 to Phase I. J Inorg Biochem 2006; 100: 1925-35.


