Anxiety and stress among science students. Study of calcium and magnesium alterations

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Abstract. Stress and anxiety of university science students (Chemistry) was evaluated in basal conditions and during exams using validated stress and anxiety questionnaires. The relations between the data obtained and various biochemical markers were established. Results showed that the evaluated students did not experience stress increase as a consequence of exams but suffered a significant increase in anxiety. The psychological findings agree with the urinary biomarkers studied. It is known that anxiety is related to partial magnesium reduction associated with a urinary magnesium excretion increase, as observed in the present data. Nevertheless, stress also correlates with a urinary calcium increase which was not detected in the present study.

Key words: stress, anxiety, psychological questionnaire, biochemical marker, magnesium, calcium

Stress is related to well established biomarkers such as cortisol [1-4] and aldosterone. The increase in intracellular cortisol with respect to aldosterone in renal cells prevents the aldosterone effect, since these two hormones compete for the same mineral-corticoid receptor [5-7]. The aldosterone suppression induces an increase in urinary calcium since this hormone increases renal calcium reabsorption by calcium channels [8].

Anxiety is associated with an increase in catecholamines [9-11] which is responsible for an increase in magnesium urinary excretion and a decrease in its plasmatic concentrations [12-16]. In fact, Mg reduction increased anxiety-related behaviour in mice [17].

Several studies have demonstrated that the daily activity of scholars causes significative stress [18-21] and this could be related to a great number of psychosomatic disorders [22, 23]. Chronic stress was found to reduce the endothelial function, which may also be associated with an intracellular magnesium level decrease in humans [24].

Scientific studies (Chemistry, Physics, Mathematics) are highly demanding and need a huge continuous effort from students. The aim of this paper was to evaluate the stress and anxiety of the students in basal conditions and during exams through validated stress and anxiety questionnaires. In addition, the correlation between the data obtained and biochemical markers such as calcium and magnesium would be established.

Material and methods

Thirty-five volunteers (12 males and 23 females aged 18 to 20 years) in the first level of university chemis-
try studies (Faculty of Sciences of the University of Balearic Islands) were selected. Written informed consent was obtained from all volunteers. The study was divided into two parts. The first one corresponded to basal conditions and was developed at the beginning of the academic course (beginning of November) and the second during exams in the middle of the academic course (end of January). In all cases stress and anxiety questionnaires and urine analysis were performed.

### Stress and anxiety questionnaires

Validated stress and anxiety questionnaires were used. STAI (State Treats Anxiety Inventory) questionnaire [25] was used to evaluate state (STAIE) and trait (STAIR) anxiety. PSQ (Perceived Stress Questionnaire) [26] was used also to assess recent (stress) and general (stressr) stress. Both questionnaires have satisfactory validity properties [25, 26]. Internal consistency was 0.83 - 0.92 for the STAI [25] and 0.87 for the state and 0.9 for the trait PSQ [26]. The correlations between scales are shown in table 1.

### Analysis of urinary samples

Urine was analyzed in basal conditions and during exams. All subjects were on a free diet at the time of urine collection and none of them was undergoing pharmacological treatment. Twenty-four-hour urine was collected in sterile flasks containing thymol as a preservative. The volume was recorded and the samples were stored at -20°C until assayed. Two-hour urine collection was performed next day, after 24-hour urine collection and after overnight fasting, and the pH was immediately measured with a glass electrode (Crison pH-meter). Calcium, magnesium and phosphorus were determined in both samples by atomic emission spectrometry using an inductively coupled plasma (ICP Optima 5300DV) and creatinine by Sigma kit (ref. 557).

### Statistics

Values in the tables are expressed as mean ± SE. The Student t-test for paired-values was used to assess differences of means. Conventional Windows software was used for statistical analysis. A value of p < 0.05 was considered significant.

### Results

Questionnaire results are summarized in table 2. The only significant differences were observed in state anxiety as shown by STAI questionnaire in basal conditions (39.7 ± 4.3) and during exams (72.6 ± 3.5). No significant differences appeared between the stress questionnaires.

Urine sample analysis results are reported in tables 3 and 4. There were no significant differences in diuresis, pH, calcium and creatinine concentrations and excretion in 24h urine when basal values (urine collected in November) were compared with values obtained during exams (urine collected in January). Nevertheless, a significant magnesium and phosphate (phosphorus) increase were noticed in 24h urine collected during exams (94±8mg/L Mg, 929 ± 82 mg/L P) as compared with basal values (72 ± 5 mg/L Mg, 686 ± 42 mg/L P). No significant differences were observed between the 2h urinary parameters (diuresis, pH, Mg, P, creatinine) in both groups, except for calcium excretion and concentrations.

### Discussion

The present results showed that the evaluated students did not experiment a stress increase during

<table>
<thead>
<tr>
<th>Condition</th>
<th>Questionnaire</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stress</td>
<td>stressr</td>
<td>STAIE</td>
<td>STAIR</td>
</tr>
<tr>
<td>Basal conditions</td>
<td>0.40 ± 0.01</td>
<td>0.38 ± 0.01</td>
<td>39.7 ± 4.3</td>
<td>43.9 ± 4.9</td>
</tr>
<tr>
<td>During exams</td>
<td>0.39 ± 0.01</td>
<td>0.40 ± 0.02</td>
<td>72.6 ± 3.5*</td>
<td>49.4 ± 4.7</td>
</tr>
</tbody>
</table>

* p < 0.001 versus basal conditions.

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**Table 1.** Correlations between scales [26].

<table>
<thead>
<tr>
<th></th>
<th>General PSQ</th>
<th>Recent PSQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>State anxiety (STAIE)</td>
<td>0.22</td>
<td>0.28</td>
</tr>
<tr>
<td>Trait anxiety (STAIR)</td>
<td>0.65</td>
<td>0.69</td>
</tr>
</tbody>
</table>

**Table 2.** Anxiety and stress questionnaires texts results under basal conditions and during exams (n = 35).
exams but suffered a significant anxiety increase. It is interesting to observe that the psychological findings agree with urinary biomarkers studied. It is known that anxiety is related to partial magnesium decrease associated with an increase in urinary magnesium excretion [12-17]. This might be partially attributed to the plasmatic glucose decrease caused by anxiety that leads to catecholamine secretion in order to restore glucose levels. These hormones are implicated in hypomagnesemia [9-11]. Also, an increase in aldosterone secretion might be able to explain the findings of this paper, as aldosterone leads to an increased renal excretion of magnesium [27-29].

The noticeable increase in muscular tension linked to anxiety consumes an important amount of energy that is partially due to the ATP-ADP transformation. A high increase in urinary phosphate excretion [30-32] also contributes to the magnesium reduction. Indeed, magnesium has been proposed for treatment in different anxiety disorders [33-38].

Stress correlates with a urinary calcium increase resulting from cortisol liberation [39, 40]. Cortisol blocks the calcium tubular reabsorption mediated by aldosterone, and as a consequence increases calcium urinary excretion [5-8]. Moreover, aldosterone causes an increase in renal magnesium excretion. In the present paper, we did not observe changes in stress and no correlatively significant increases were detected in urinary calcium concentration or excretion.

It must be pointed out that no differences were observed in urinary concentrations and excretion of magnesium and phosphate (phosphorus) when the first urine of the morning was studied (2h urine). This can be linked to the relaxation induced by sleep that must be accompanied by a decrease in anxiety.

### Table 3. Urinary biomarkers determined in 24h urine under basal conditions and during exams (n = 35).

<table>
<thead>
<tr>
<th>Urinary parameter</th>
<th>Basal conditions</th>
<th>During exams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>1318 ± 88</td>
<td>1213 ± 74</td>
</tr>
<tr>
<td>pH</td>
<td>6.3 ± 0.1</td>
<td>6.4 ± 0.1</td>
</tr>
<tr>
<td>[Calcium] (mg/L)</td>
<td>131 ± 11</td>
<td>145 ± 12</td>
</tr>
<tr>
<td>Calcium excretion (mg)</td>
<td>155 ± 10</td>
<td>173 ± 19</td>
</tr>
<tr>
<td>[Magnesium] (mg/L)</td>
<td>72 ± 5</td>
<td>94 ± 8*</td>
</tr>
<tr>
<td>Magnesium excretion (mg)</td>
<td>84 ± 4</td>
<td>121 ± 19</td>
</tr>
<tr>
<td>[Phosphorus] (mg/L)</td>
<td>686 ± 42</td>
<td>929 ± 82*</td>
</tr>
<tr>
<td>Phosphorus excretion (mg)</td>
<td>808 ± 44</td>
<td>1180 ± 156*</td>
</tr>
<tr>
<td>[Creatinine] (mg/L)</td>
<td>915 ± 89</td>
<td>1002 ± 82</td>
</tr>
</tbody>
</table>

* p < 0.05 versus basal conditions.

### Table 4. Urinary biomarkers determined in 2h urine under basal conditions and during exams (n = 35).

<table>
<thead>
<tr>
<th>Urinary parameter</th>
<th>Basal conditions</th>
<th>During exams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>105 ± 9</td>
<td>89 ± 10</td>
</tr>
<tr>
<td>pH</td>
<td>5.9 ± 0.1</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td>[Calcium] (mg/L)</td>
<td>149 ± 14</td>
<td>115 ± 10*</td>
</tr>
<tr>
<td>Calcium excretion (mg)</td>
<td>15 ± 2</td>
<td>9 ± 1*</td>
</tr>
<tr>
<td>[Magnesium] (mg/L)</td>
<td>84 ± 7</td>
<td>72 ± 4</td>
</tr>
<tr>
<td>Magnesium excretion (mg)</td>
<td>8 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>[Phosphorus] (mg/L)</td>
<td>793 ± 87</td>
<td>750 ± 72</td>
</tr>
<tr>
<td>Phosphorus excretion (mg)</td>
<td>72 ± 10</td>
<td>69 ± 13</td>
</tr>
<tr>
<td>[Creatinine] (mg/L)</td>
<td>1562 ± 182</td>
<td>1507 ± 141</td>
</tr>
</tbody>
</table>

* p < 0.05 versus basal conditions.
Conclusion
The present results showed that the evaluated university science students did not experience stress increase as a consequence of exams but suffered a significant increase in anxiety. This was associated to a urinary magnesium excretion increase responsible for partial magnesium depletion.

Acknowledgements
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