Clinical paper

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

G. Grases[1], J.A. Pérez-Castelló[1], P. Sanchis[2], A. Casero[1], J. Perelló[2], B. Isern[2], E. Rigo[1], F. Grases[2]

[1] *University of Balearic Islands, Dept. Applied Pedagogy and Educational Psychology, Palma of Mallorca, Spain;* [2] *University of Balearic Islands, University Institute of Health Sciences Research (IUNICS), Palma of Mallorca, Spain*

Correspondence: F. Grases, Laboratory of Renal Lithiasis Research, Faculty of Sciences, University of Balearic Islands, 07122 Palma of Mallorca, Spain.
<fgrases@uib.es>

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 mineralcorticoid 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-

Table 1. Correlations between scales [26].

	General PSQ	Recent PSQ
State anxiety (STAI) (n = 80)	0.22	0.28
Trait anxiety (STAI) (n = 80)	0.65	0.69

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 ± 8 mg/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 2. Anxiety and stress questionnaires texts results under basal conditions and during exams (n = 35).

Condition	Questionnaire			
	stress	stressr	STAIE	STAIR
Basal conditions	0.40 ± 0.01	0.38 ± 0.01	39.7 ± 4.3	43.9 ± 4.9
During exams	0.39 ± 0.01	0.40 ± 0.02	72.6 ± 3.5*	49.4 ± 4.7

* $p < 0.001$ *versus* basal conditions.

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

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

* $p < 0.05$ *versus* basal conditions.

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

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

* $p < 0.05$ *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

P.S. expresses her appreciation to the Spanish Ministry of Education, Culture and Sport for a fellowship of the FPU program. Also, B.I. expresses his appreciation to the Conselleria d'Innovació i Energia del Govern de les Illes Balears for a fellowship. This work was supported by the Conselleria d'Innovació i Energia del Govern de les Illes Balears (Grant PROIB-2002GC1-04) and by the project BQU 2003-01659 of the Spanish Ministry of Science and Technology.

References

1. Collins A, Frankenhaeuser M. Stress responses in male and female engineering students. *J Human Stress* 1978; 4: 43-8.
2. Carpenter Jr. WT, Gruen PH. Cortisol's effects on human mental functioning. *J Clin Psychopharmacol* 1982; 2: 91-101.
3. Fredrikson M, Sundin O, Frankenhaeuser M. Cortisol excretion during the defense reaction in humans. *Psychosom Med* 1985; 47: 313-9.
4. Burke HM, Davis MC, Otte C, Mohr DC. Depression and cortisol responses to psychological stress: a meta-analysis. *Psychoneuroendocrinology* 2005; 30: 846-56.
5. Ferrari P, Bianchetti MG, Sansonnens A, Frey FJ. Modulation of Renal Calcium Handling by 11-Hydroxysteroid Dehydrogenase Type 2. *J Am Soc Nephrol* 2002; 13: 2540-6.
6. Ferrari P. Cortisol and the renal handling of electrolytes. Role in glucocorticoid-induced hypertension and bone disease. *Best Pract Res Clin Endocrinol Metab* 2003; 17: 575-89.
7. Reid IR, Ibbertson HK. Evidence for decreased tubular reabsorption of calcium in glucocorticoid-treated asthmatics. *Horm Res* 1987; 27: 200-4.
8. Leclerc M, Brunette MG, Couchourel D. Aldosterone enhances renal calcium reabsorption by two types of channels. *Kidney Int* 2004; 66: 242-50.
9. Keenan D, Romani A, Scarpa A. Regulation of Mg^{2+} homeostasis by insulin in perfused rat livers and isolated hepatocytes. *FEBS Lett* 1996; 395: 241-4.
10. Torres LM, Youngner J, Romani A. Role of glucose in modulating Mg^{2+} homeostasis in liver cells from starved rats. *Am J Physiol Gastrointest Liver Physiol* 2005; 288: G195-G206.
11. Ueshima K, Tachibana H, Suzuki T, Hiramori K. Factors affecting the blood concentration of ionized magnesium in patients in the acute phase of myocardial infarction. *Heart Vessels* 2004; 19: 267-70.
12. Durlach J, Pages N, Bac P, Bara M, Guiet-Bara A, Agrapart C. Chronopathological forms of magnesium depletion with hypofunction or with hyperfunction of the biological clock. *Magnes Res* 2002; 15: 263-8.
13. Durlach J, Pages N, Bac P, Bara M, Guiet-Bara A. Importance of magnesium depletion with hypofunction of the biological clock in the pathophysiology of headaches with photophobia, sudden infant death and some clinical forms of multiple sclerosis. *Magnes Res* 2004; 17: 314-26.
14. Durlach J, Pages N, Bac P, Bara M, Guiet-Bara A. Magnesium depletion with hypo- or hyper-function of the biological clock may be involved in chronopathological forms of asthma. *Magnes Res* 2005; 18: 19-34.
15. Fromm L, Heath DL, Vink R, Nimmo AJ. Magnesium attenuates post-traumatic depression/anxiety following diffuse traumatic brain injury in rats. *J Am Coll Nutr* 2004; 23: 529S-533S.
16. Kirov GK, Tsachev KN. Magnesium, schizophrenia and manic-depressive disease. *Neuropsychobiology* 1990; 23: 79-81.
17. Singewald N, Sinner C, Hetzenauer A, Sartori SB, Murck H. Magnesium-deficient diet alters depression- and anxiety-related behaviour in mice. Influence of desipramine and *hypericum perforatum* extract. *Neuropharmacology* 2004; 47: 1189-97.
18. Hess R, Copeland EP. Students stress, coping strategies, and school completion: a longitudinal perspective. *School Psychol Quart* 2001; 16: 389-405.
19. Peterson JL, Folkman S, Bakeman R. Stress, coping, HIV status, psychosocial resources, and depressive mood in African American gay, bisexual, and heterosexual men. *Am J Community Psychol* 1996; 24: 461-87.
20. Roberts SM. Applicability of the goodness-of-fit hypothesis to coping with daily hassles. *Psychol Rep* 1995; 77: 943-54.
21. Ruffin CL. Stress and health: little hassles *versus* major life events. *Aust Psychol* 1993; 28: 201-8.
22. Aro H, Paronen O, Aro S. Psychosomatic symptoms among 14-16 year old Finnish adolescents. *Soc Psychiatry* 1987; 22: 171-6.
23. Goodman JE, McGrath PJ. The epidemiology of pain in children and adolescents: a review. *Pain* 1991; 46: 247-64.
24. Takase B, Akima T, Uehata A, Ohsuzu F, Kurita A. Effect of chronic stress and sleep deprivation on both flow-mediated in the brachial artery and the intracellular magnesium level in humans. *Clin Cardiol* 2004; 27: 223-7.
25. Spielberger CD, Gorsuch RL, Lushene RE. *State-Trait Anxiety Inventory Questionnaire*. Consulting Psy-

chologists Press, Inc, California, 1968. Validated by Dept. I + D TEA Editions S.A., Madrid, 1982.

26. Sanz-Carrillo C, García-Campayo J, Rubio A, Santed MA, Montoso M. Validation of the Spanish version of the Perceived Stress Questionnaire. *J Phychosom Res* 2002; 52: 167-72.
27. Murck H. Magnesium and affective disorders. *Nutr Neurosci* 2002; 5: 375-89.
28. Ferrari P. Cortisol and the renal handling of electrolytes: role in glucocorticoid-induced hypertension and bone disease. *Best Pract Res Clin Endocrinol Metab* 2003; 17: 575-89.
29. Emanuele E, Geroldi D, Minoretti P, Coen E, Politi P. Increased plasma aldosterone in patients with clinical depression. *Arch Med Res* 2005; 36: 544-8.
30. Balon R, Yeragani VK, Pohl R. Relative hypophosphatemia in patients with panic disorder. *Arch Gen Psychiatry* 1988; 45: 294-5.
31. Maddock RJ, Moses Jr. JA, Roth WT, King R, Murchison A, Berger PA. Serum phosphate and anxiety in major depression. *Psychiatry Res* 1987; 22: 29-36.
32. Shioiri T, Kato T, Murashita J, Hamakava H, Inubushi T, Takahashi S. High-energy phosphate metabolism in the frontal lobes of patients with panic disorder detected by phase-encoded 31P-MRS. *Biol Psychiatry* 1996; 40: 785-93.
33. Bockova E, Hronek J, Kolomaznik M, Polackova J, Curdova NO. Potentiation of the effects of anxiolytics with magnesium salts. *Cesk Psychiatr* 1992; 88: 141-4.
34. Ezhov AV, Pimenov LT. Effect of adjuvant magnesium therapy on the quality of life and emotional status of elderly patients with stable angina. *Adv Gerontol* 2002; 10: 95-8.
35. Grazzi L, Andrasik F, Usai S, Bussone G. Magnesium as a treatment for paediatric tension-type headache: a clinical replication series. *Neurol Sci* 2005; 25: 338-41.
36. Hanus M, Lafon J, Mathieu M. Double-blind, randomised, placebo-controlled study to evaluate the efficacy and safety of a fixed combination containing two plant extracts (*Crataegus oxyacantha* and *Eschscholtzia californica*) and magnesium in mild-to-moderate anxiety disorders. *Curr Med Res Opin* 2004; 20: 63-71.
37. Poleszak E, Szewczyk B, Kedzierska E, Wlaz P, Pilc A, Nowak G. Antidepressant- and anxiolytic-like activity of magnesium in mice. *Pharmacol Biochem Behav* 2004; 78: 7-12.
38. Recarte-Garcia C, del Castillo-Rueda A, Torres-Segovia F. Anxiolytic effect of magnesium. *An Med Interna* 1991; 8: 576.
39. Goldsmith RS. Calcium metabolism under stress and in repose. *Life Sci Space Res* 1972; 10: 87-101.
40. Najem GR, Seebode JJ, Samady AJ, Feuerman M, Fierman L. Stressful life events and risk of symptomatic kidney stones. *Int J Epidemiol* 1997; 26: 1017-23.