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Oral Magnesium Supplementation and Test Anxiety in University Undergraduates

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Magnesium (Mg++) has significant potential in the treatment of test anxiety. This study assessed the possibility that oral Mg++ could reduce test anxiety, through the reversal of stress-induced hypomagnesic states that dysregulate the autonomic stress response. Each participant completed two measures of anxiety, and received a five-day course of oral Mg++ (300 mg/day) or matched placebo before the beginning of final exams. Before their first exam, each participant completed the Spielberger State Anxiety Inventory. The level of self-reported anxiety before final exams did not differ between the placebo and Mg++ groups (p = 0.69). A number of possible factors could have produced this result, and future research needs to be conducted to determine the specific cause of this finding.

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Barlow (2000) has defined anxiety as "a future-oriented mood state in which one is ready or prepared to attempt to cope with upcoming negative events" (p. 1249). Anxiety disorders affect approximately 40 million Americans a year (National Institutes of Mental Health, 2009), and result from maladaptive changes in the central nervous system that produce hyperexcitability of the hypothalamic-pituitary-adrenocortical (HPA) axis (Holmes, Heilig, Rupniak, Steckler, & Griebel, 2003). The neuroplastic changes assumed to underlie these changes are mediated primarily by excessive glutamatergic activity (Bergink, van Megen, & Westenberg, 2004; Timmermans, Xiong, Hoogenraad, & Krugers, 2013), and current pharmacological treatments for anxiety (such as benzodiazepines and serotonin selective reuptake inhibitors (SSRIs)) attempt to suppress this neuronal overexcitation (Lakhan & Vieira, 2010). Benzodiazepines act as noncompetitive GABA-A agonists, and thus produce an anxiolytic effect through a hyperpolarization of the postsynaptic neurons in the limbic system (Feldman, Meyer, & Quenzer, 1997). Benzodiazepines are less than ideal as therapeutic tools in the long term treatment of anxiety, as they can produce states of dependence as well as cognitive impairment that can persist long after use has ended (Stewart, 2005). The other major pharmacological treatment for anxiety, SSRIs, typically require several weeks to become effective and often have low efficacy rates (Eby & Eby, 2010). SSRI's can also induce several negative side effects including: suicidality, decreased alertness, sleep disturbances, weight gain, sexual dysfunction, and dependency (Feldman et al., 1997).

The negative side effects of current medications prescribed for anxiety expose an avenue for discovering anxiolytics with high therapeutic efficacy and a reduced chance of potential harmful side effects. On such class of pharmacological treatments are HPA axis targeted therapies that interfere with the normal functions of NMDA receptors (Bergink, et al., 2003). Unfortunately, common NMDA receptor antagonists (such as dextromethorphan, ketamine, and PCP) have their own undesirable set of side effects which have largely prevented their clinical use as anxiolytics (Bermudo-Soriano, Perez-Rodriguez, Vaquero-Lorenzo, & Baca-Garcia, 2012). However, significant interest has developed around the potential anxiolytic properties of elemental magnesium (Mg++).

Mg++ is an essential cation utilized in the human body for over 300 biochemical reactions, and is one of the most common cations in the brain (Laaraker, van Lith, & Ohl, 2011; National Institutes of Health, 2009). The functions of Mg++ are varied, and include protecting biological membranes, modulating cellular transport functions and receptors, involvement in DNA replication, and aiding in signal transduction and protein synthesis (Sissi & Palumbo, 2009; Topf & Murray, 2003;). Mg++ has variety of roles within the central nervous system, including acting as an enzymatic cofactor in neurotransmitter synthesis, functioning as a calcium (Ca++) agonist which causes biochemical and structural changes in neurons, and inhibiting NMDA receptor activity (Laaraker, et al., 2011).

Activity at NMDA receptors alters neurons (particularly synaptic and dendritic spine modification) via second messenger mediated changes induced by Ca++ currents into the cell (Feldman et al., 1997). NMDA receptors are unique in that their central ion channel is blocked by a Mg++ ion, and a simultaneous binding of ligands (glutamate and a co-agonist, glycine or D-serine) from the pre-synaptic cell as well as a sufficient depolarization of the post-synaptic neuron is required to remove this Mg++ ion (Feldman et al., 1997).

This removal of the Mg++ from the ion channel pore of the receptor occurs via a voltage-dependent change in the affinity of Mg++ to its binding site (Feldman et al., 1997). Once the Mg++ is removed from the channel pore, Ca++ and sodium (Na+) can enter the cell (Feldman et al., 1997). These Na+ currents serve to further depolarize the cell while Ca++ currents induce a wide variety of cellular processes that are mediated by various second messengers (Feldman et al., 1997). In this sense, free elemental Mg++ can act as a potent and voltage-dependent inhibitor of NMDA activity (Murck, 2002).

In chronic anxiety, there is an abnormal increase in the sensitivity of the excitatory stress response (Lee & Goto, 2011; Martin et al., 2011), and this increase is the result of heightened glutamatergic activity within the hypothalamic-pituitary-adrenocortical axis (HPA; Kuzmiski, 2010; Ziegler, Edwards, Ulrich-Lai, Herman, & Cullinan, 2012). NMDA receptors in the limbic system (and ventral hippocampus, specifically) appear to play an important role in the activation of the HPA axis, and are also fundamentally involved in anxiety, fear-conditioning, and inhibitory-avoidance behaviors (Barkus et al., 2010; Bergink et al., 2004; Bermudo-Soriano et al., 2012). Given the central role played by Mg++ in NMDA receptor function, it is reasonable to assume that changes in systemic Mg++ levels may alter NMDA activity, and, by extension, the degree of activation of the HPA axis. Indeed, elevated levels of catecholamine neurotransmitters and corticosteroids (all of which are released at increased rates when the HPA axis is active) are associated with increased urinary Mg++ excretion and decreased blood serum Mg++ (Seelig, 1994). Low Mg++ levels have also been associated with, and implicated in, elevated states of anxiety and stress in numerous investigations using both human and animal models (De Souza, Walker, Robinson, & Bolland, 2000; Eby & Eby, 2006; Grases, et al., 2006; Hanus, Lafon, & Mathieu, 2004; Laarakker, et al., 2011; Lakhan & Vieira, 2010; Poleszak, 2008; Poleszak, Szewczyk, Kedzierska, Wlaz, Pilc, & Nowak, 2004; Poleszak, Wlaz, Wrobel, Fidecka, & Nowak, 2008; Sartori, Whittle, Hetzenauer, & Singewald, 2012; Seelig, Berger, & Spielholz, 1975; Seelig, 1994; Singewald, Sinner, Hetzenauer, Sartori, & Murck, 2004).

Levels of Mg++ in the brain are tightly regulated, and are relatively resistant to dietary depletion (Murck, 2002). However, this regulation appears to involve an adrenergic mechanism whereby elevations in adrenaline or the administration of an adrenergic agonist promotes increased Mg++ excretion (Murck, 2002). Therefore, increases in sympathetic nervous system arousal may lead to increased urinary Mg++ excretion and decreased plasma concentrations in otherwise replete individuals (Grases et al., 2006; Murck, 2002). Such a stress-related increase in Mg++ excretion could initiate a positive feedback loop whereby increasing Mg++ excretion would further drive a transient hypomagnesemic state, resulting in a further increase in the HPA/sympathetic nervous system stress response and additional Mg++ excretion (Seelig, 1994). The existence of such a feedback loop is supported by numerous cellular homeostatic changes that produce hypersensitivity and dysregulation in the HPA axis following long-term exposure to stress (Murck, 2002). These changes have been correlated with the symptomology of several neuropsychiatric disorders including chronic anxiety, bipolar disorder, and depression (Eby & Eby, 2010; Murck, 2002; Sartori et al., 2012).

To summarize, Mg++ functions as an inhibitor of NMDA receptors, and through this action, Mg++ reduces HPA activity, which is responsible for facilitating the stress response (Topf & Murray, 2003). The neurobiological functions of NMDA receptors

indicate that a targeted antagonism of these receptors may reduce anxiety and related negative behavioral changes (Bermudo-Soriano et al., 2012). Decreases in Mg++ levels have been shown to be associated with an increase in the production of corticosteroids and catecholamines, which implicates a deficiency in CNS Mg++ in the production of states of stress and anxiety (Murck, 2002). It appears that during times of stress and anxiety, healthy individuals who would otherwise be Mg++ replete can suffer from transient Mg++ deficiencies, as indicated by increased Mg++ excretion (Grases et al., 2006; Murck, 2002). The potential application of oral Mg++ as an anxiolytic has not been widely investigated although this possibility has been suggested in multiple studies (Lakhan & Viera, 2010; Murck, 2002).

Test anxiety can be extremely detrimental to university student performance. Given the role of high-stakes standardized tests (such as the GRE) in graduate admissions, as well as the role that exams play in the grading of most university courses, the negative effects of test anxiety on university student performance are of major concern. For college students, final exams are typically a temporary (rather than chronic) stressor. As discussed above, HPA axis activation during final exams can cause a transient Mg++ deficiency in otherwise Mg++ replete individuals (Grases et al., 2006). In a study of university students, Grases et al. (2006) found that increases in anxiety during exams correlated with elevated urinary Mg++ excretion that was concurrent with a decrease in blood plasma Mg++ concentrations. Such a temporary hypomagnesemic state may likely result in the increased activation of NMDA receptors, and therefore increased sensitivity of the HPA, further increasing the stress response in these individuals.

During the transient Mg++ depletion caused by final exams for college students, an external dietary supplementation of Mg++ may serve to decrease the hypersensitivity of the HPA through reduction of the NMDA receptor activation. This study utilizes a double-blind placebo-controlled design comparing the effects of Mg++ citrate versus a gelatin placebo in reducing test anxiety in university students. This research hoped to demonstrate the potential application of Mg++ as an alternative anxiolytic by decreasing the hypersensitive state of the HPA during times of high stress. Magnesium citrate was chosen because it possesses favorable bioavailbility relative to several more commonly used inorganic salts, such as magnesium oxide (Coudray et al., 2005; Firoz & Graber, 2001; Ranade & Somberg, 2001; Walker, et al., 2003).

Method

The experimental protocol was approved by the Institutional Review Board of Elon University, and all procedures were in accordance with the Helsinki Declaration of 1975, as revised in 1983. A convenience (quasi-random) sample of 147 university undergraduate volunteers was recruited from undergraduate classes at a university in the southeast United States using an online human subject pool management system (SONA, Sona Systems, Tallinn, Estonia) and word of mouth. No participants were excluded from this study based upon preexisting conditions or potential medication interactions. Some students received course credit for participation; others were entered into a raffle to win one of three $15 Target gift cards.

This study utilized a standard randomized double-blind, placebo controlled design. The participants attended an initial appointment at least a week before their first university final exam (for the relevant semester in which study participation occurred) that lasted less

than 15 minutes. During this appointment the participants gave written informed consent and were assigned a participant identification number (PID), ascending in order of study enrollment. Demographic information was gathered on the participant (age in years, self-reported biological sex, and self-reported ethnicity). The researchers measured participants' height (in cm) and weight (in kg) using a stadiometer and digital scale (Health o meter, Boca Raton, FL, USA). Additionally, the participants were left alone to complete, in their own time, two self-report assessments of anxiety. The first assessment was the Westside Test Anxiety Scale (WTAS), an anxiety scale consisting of ten test/exam-related statements, where the participants rank, using a 5 point scale, "how true each of the following is of you, from extremely or always true, to not at all or never true" (Driscoll, 2007). The second assessment was the Adult Manifest Anxiety Scale-College (AMAS-C; Reynolds, Richmond, & Lowe, 2003). The AMAS-C is a 49 item scale that asks participants to respond to statements by circling "Yes" for if they think the statement is mostly true about them or "No" if they think it is mostly false--for example: "Others seem to do things more easily than I can." (Reynolds et al., 2003). The AMAS-C was included in the study protocol (in addition to the WTAS) because the WTAS was validated using data gathered from a small sample of college students (Driscoll, 2007), and we were curious if the outcomes generated by this assessment would correlate with those produced by the more widely used AMAS-C.

The participants were given an opaque medicine bottle containing 15 unmarked capsules (all either Mg++ or placebo) and were provided both written and oral instructions to consume one capsule, three times a day with meals or food, for five days prior to their first final exam of the semester. Each placebo (PBO) capsule contained 650 mg of food grade gelatin (Puritan's Pride Inc., Oakdale NY, USA) and each Mg++ capsule contained 100 mg of Mg++ (as magnesium citrate; Puritan's Pride Inc., Oakdale NY, USA). The Mg++ and placebo capsules were visually indistinguishable. Assignment to capsule group was made based on whether the participant's randomly assigned ID number was even or odd. Participants received daily email reminders to consume their capsules, and were asked to avoid heavy consumption of whole grain breads, cereals, and mineral waters (all of which typically contain high levels of Mg++) in the five days prior to their first exam.

On the evening before their first final exam each participant completed the current state component of the Spielberger State Anxiety Inventory (Form Y-1; Spielberger, 1983). This instrument was emailed to the participants and administered through a Google Form. The responses were returned via email. After data collection was completed, WTAS scores were utilized to create four groups based categorical criteria for scores generated by the WTAS (Driscoll, 2007). Approximating these categories, WTAS scores were ranked as follows: Group 1, "normal anxiety" (WTAS < 2.5); Group 2, "elevated normal anxiety" (WTAS >= 2.5 and < 3.0); Group 3, "high anxiety" (WTAS >=3.0 and < 4.0); and, Group 4, "very high anxiety" (WTAS >=4.0).

The potential effect of Mg++ on SSAI state anxiety scores was assessed via an analysis of covariance (ANCOVA), constructed using SAS 9.3 for Windows (SAS Institute, Cary NC, USA). This ANCOVA model included treatment condition (PBO or Mg++), self-reported sex, body weight (in kg), WTAS rank, and the interactions of; 1) drug and sex and, 2) drug and WTAS rank. Model diagnostics were carried out by examining plots of model residuals. An alpha level of 0.05 was used for all tests.

Results

A total of 147 individuals participated in this study. However, the data from only 122 participants were included in the analysis, as 25 individuals failed to complete the SSAI. This remaining participant group consisted of 31 males and 91 females. Participants ranged in age from 18 to 22 years (M = 19.3 years; SD = 1.17), and were of generally average weight (M = 67.23 kg; SD = 14.27) and BMI (M = 23.33; SD = 3.96). Of the 122 participants, 96 (78.7%) self-identified as "White/Caucasian". No participants withdrew from the study or reported adverse reactions, and all participants verbally reported to have followed the capsule administration and testing instructions as described earlier. A total of 60 participants received the PBO (15 males, 45 females) and 62 (16 males, 46 females) received the Mg++.

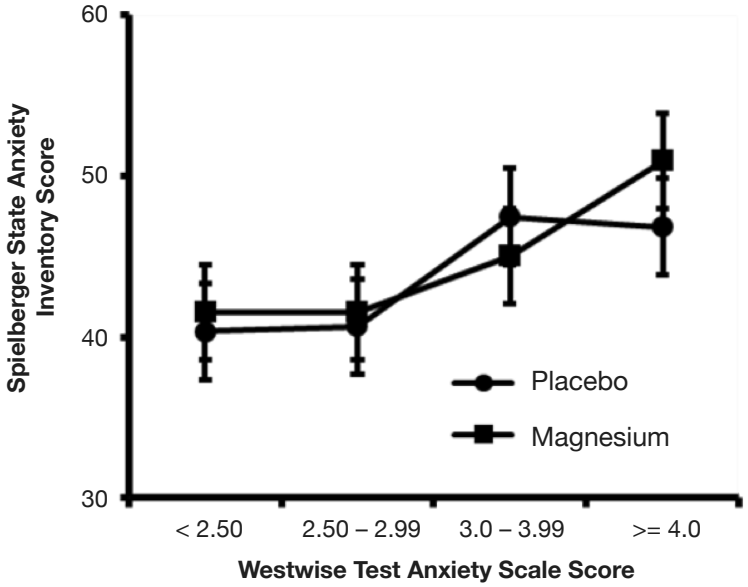
WTAS scores were approximately normally distributed in the sample across the range of values for the scale (M = 2.90; SD = 0.74; min = 1.5; max = 4.8). WTAS scores were mirrored by the test anxiety T scores from the AMAS-C, which were also approximately normally distributed (M = 54.08; SD = 9.04; min = 32; max = 77). The method of ranking the WTAS scores (as described in the Methods) produced WTAS group n's as follows: Group 1: n = 34, Group 2: n = 38, Group 3: n = 38, Group 4: n = 12. SSAI scores were approximately normally distributed across the range of values for this scale (M = 43.5; SD = 10.7, min = 20, max = 70). SSAI scores were strongly positively correlated with both WTAS scores (r = 0.28, p = 0.002) and test anxiety T scores from the AMAS-C (r = 0.26, p = 0.004). WTAS scores and test anxiety T scores from the AMAS-C were also strongly correlated with each other (r = 0.71, p < .0001).

In the ANCOVA analysis (described above), the main effect of WTAS rank was significant (F(3,111) = 3.37, p = 0.02, classical η^2 = 0.074). WTAS rank groups 1 and 2 were not significantly different from each other (p = 0.95), groups 3 and 4 also were not different (p = 0.44). However, significant differences existed between WTAS rank groups 1 and 3 (p = 0.04), 2 and 3 (p = 0.03), 1 and 4 (p = 0.02) and 2 and 4 (p = 0.02), in all cases demonstrating that individuals with "high" or "very high" test anxiety indicated significantly higher state anxiety on the SSAI during final exams. The main effects of treatment condition (F(1,111) = 0.16, p = 0.69, classical η2 = 0.001), and the interactions of treatment and sex (F(1,111) = 0.88, p = 0.35, classical η2 = 0.006) and treatment and WTAS rank (F(3,111) = 0.40, p = 0.75, classical η^2 =0.009) were all not significant; collectively indicating that Mg++ treatment had no significant effect on state anxiety related to final exams (Figure 1). The main effects of self-reported sex (F(1,111) = 0.00, p = 0.97, classical η^2 < 0.0001) and body weight (F(1,111) = 2.61, p = 0.11, classical η^2 = 0.019) were also not significant. Identical conclusions were drawn from a second ANCOVA analysis that that collapsed WTAS groups 1 and 2 into one group and 3 and 4 into a second group.

Discussion

The existence of multiple negative side effects associated with the use of many current anxiolytics (such as benzodiazepines) emphasizes the importance of identifying new anti-anxiety medications. In this regard, HPA axis targeted pharmacotherapies that interfere with the normal functions of NMDA receptors hold considerable promise (Bergink et al., 2003). Unfortunately, common NMDA antagonists (such as dextromethorphan, ketamine, and PCP) have their own undesirable set of side effects which have largely prevented their clinical use as anxiolytics (Bermudo-Soriano et al., 2012). Because elemental Mg++ functions to antagonize NMDA activity, considerable interest exists regarding magnesium's

Figure 1: *Participant's self-reported state anxiety (measured using the SSAI) in relation to each participant's score on the Westside Test Anxiety Scale for the placebo and magnesium groups. Neither the main effect of treatment ($p = 0.69$, classical $\eta 2 = 0.001$) nor the interaction of treatment and WTAS scores ($p = 0.75$, classical $\eta^2 = 0.009$) were significant.*



potential anxiolytic properties (Bermudo-Soriano et al., 2012).

This research hoped to demonstrate the potential application of Mg++ as an alternative anxiolytic to treat test anxiety in university students by decreasing the hypersensitive state of the HPA during times of high stress. Oral Mg++ treatment appeared to have had no significant effect on state anxiety related to taking final exams in a university setting. The ineffectiveness of the Mg++ could have been caused by a variety of factors including the choice of Mg++ citrate as the specific preparation, dose and/or number of days administered, or a general lack of anxiolytic properties of Mg++.

Mg++ citrate was chosen because of its favorable bioavailability relative to inorganic salts, such as Mg++ oxide (Coudray et al., 2005; Firoz & Graber, 2001; Ranade & Somberg, 2001; Walker, et al., 2003). Available Mg++ preparations include oxide, citrate, chloride, aspartate, and lactate, each of which vary widely in elemental Mg++ concentration and absorption rate (National Institutes of Health, 2009). A common side effect of oral Mg++ supplementation is osmotic diarrhea, and the choice of a 300mg/day dose of Mg++ citrate was motivated by an attempt to minimize gastrointestinal upset in our participants (Topf & Murray, 2003). Common over-the-counter Mg++ preparations range from 100 mg to 500 mg—with the daily recommended Mg++ intake being 310 mg per day for females and 400 mg per day for males (National Institutes of Health, 2009). The existent literature provides little guidance regarding what an appropriate dose of Mg++ might be, so we chose 300 mg because it was near the recommended daily allowance provided by the NIH, but well below the dose that would result in gastrointestinal upset. It will be up to future studies to determine if an anxiolytic effect of oral Mg++ citrate can be achieved with a larger daily dose.

Although the failure of Mg++ citrate to produce significant anxiolytic effects

in this study could be resultant from the choice of preparation or dose, the timeline of consumption (3 times per day for 5 days) may also have been insufficient to effectively reduce test anxiety. Perhaps significant results would have been observed if a longer time course of administration was followed.

It is also theoretically possible that our results were the product of Mg++ not actually having any anxiolytic properties whatsoever. However, this seems unlikely as several human and animal studies have demonstrated significant anxiolytic effects following Mg++ supplementation (De Souza, et al., 2000; Eby & Eby, 2006; Hanus, Lafon, & Mathieu, 2004; Lakhan & Viera, 2010; Poleszak, 2008; Poleszak et al., 2004; Poleszak et al., 2008). Unfortunately, these previous studies provide little guidance in regards to dose selection for our work, because: 1) previous human studies have typically focused on clinical populations suffering from chronic anxiety, rather than transient, acute, and situation-specific forms such as test anxiety, and; 2) animal research in this area has used Mg++ doses that are quite high and not appropriate for human use and/or has focused on measures (such as open field and plus-maze behavior) that may not be directly translatable to test anxiety in university students.

One possible interpretation of our findings is that the simple act of orally consuming a capsule (whether Mg++ or gelatin placebo) three times a day for five days produced a measurable anxiolytic effect that was similar in both treatment groups. The design of this study would have benefited from the inclusion of a third group that received neither the placebo nor the Mg++. The inclusion of such a "true control" group would have allowed for the investigation of this possibility, which if uncovered, would be a finding of considerable interest.

The existent literature provides little information regarding possible sex differences in the anxiolytic properties of Mg++. Even though the present study did not document a significant interaction of treatment and sex, it remains an open question as to whether such treatment differences actually fail to exist. This study utilized a convenience sample with an uneven distribution of self-reported biological sex (31 males, 91 females). Because of this imbalance, our null finding regarding the treatment*sex interaction should be interpreted with caution, and awaits replication using a more balanced sample.

Although Mg++ was not shown to be effective within this particular study, the application of Mg++ as an anxiolytic compound should not be discounted. The vast array of biological functions of Mg++ within the central nervous system highlight the potential avenues for application of Mg++ supplementation as a treatment for stress, anxiety, and mood disorders. Given that Mg++ is inexpensive, widely available, and largely devoid of side effects at purported therapeutic doses, future research should continue to investigate the potential uses of Mg++ as a modifier of central nervous system activity.

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