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Serum ionized magnesium and calcium and sex hormones in healthy young men: importance of serum progesterone level

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Objective: To determine the serum concentrations of the sex hormones with respect to the concentrations of the biologically active fractions of magnesium (Mg) and calcium (Ca) in healthy young men and to compare them with those in young and older women.

Design: Controlled clinical study.

Setting: An academic research environment.

Patient(s): Twenty-five healthy young male volunteers.

Intervention(s): None.

Main Outcome Measure(s): Concentrations of the sex steroid hormones testosterone, estrogen, and progesterone, and levels of ionized Ca (Ca^{2+}) and Mg (Mg^{2+}) were measured in the serum of healthy young men. These levels were compared with those in young women at different phases of the menstrual cycle and with those in older women.

Result(s): The Mg^{2+} and total Mg concentrations in young men were not different from those during the follicular phase in young women or from the mean concentrations in menopausal women. The Ca^{2+} levels in young men were similar to the levels in young women during the follicular phase but significantly lower than the levels in older women. The Mg^{2+} concentration in the young men was directly and significantly related to the progesterone level, and the Ca^{2+}/Mg^{2+} ratio was inversely related to the progesterone level.

Conclusion(s): Progesterone may be a more important steroid hormone in men than previously believed. (Fertil Steril® 1999;72:817–22. ©1999 by American Society for Reproductive Medicine.)

Key Words: Testosterone, estrogen, progesterone, ionized magnesium, ionized calcium, Ca^{2+}/Mg^{2+} ratio

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It is well known that young women are protected from cardiovascular disease compared with men during their child-bearing years and that this protection is lost and even reversed after menopause (1). The literature abounds with examples citing the female hormone estrogen as the prime protector of young women, not only from cardiovascular disease but also from osteoporosis and Alzheimer's disease (1–3).

It also is known that free ionized calcium (Ca^{2+}) and free ionized magnesium (Mg^{2+}) may have important salutary or deleterious effects, depending on the concentrations circulating in the blood (4). In studying young women, older women, and young men, we closely examined the relation between these important divalent cations in their ionized forms and the sex hormones.

We recently examined serum Mg^{2+} and Ca^{2+} concentrations in healthy women of reproductive age in conjunction with their hormone profiles. We demonstrated that these women showed recurring changes in serum levels of Mg^{2+} and total Mg, and alterations in the ratio of Ca^{2+} to Mg^{2+} as serum levels of estrogen, testosterone, and progesterone rose and fell (5). With low serum levels of estrogen and testosterone, both Mg^{2+} and total Mg were comparatively elevated.

As the estrogen level rose, the Mg^{2+} level fell and the Ca^{2+} level rose; the rise in Ca^{2+} with a rise in estrogen had been demonstrated previously (6). When the progesterone level peaked, the Ca^{2+}/Mg^{2+} ratio was significantly increased (5). We also studied 25 menopausal and postmenopausal women and found that their concentrations of Mg^{2+} and total Mg were inversely related to the concentrations of estrogen circulating in their blood (7). When we compared their mean values with those of younger women at different stages in their menstrual cycles, we found that Mg^{2+} and total Mg in the menopausal women were similar to Mg^{2+} and total Mg during the early follicular phase (i.e., when serum estrogen and progesterone levels are low) in the premenopausal women. We also found that the mean serum Mg^{2+} level in the older women was significantly higher than that in the young women when serum estrogen and progesterone peaked. Finally, Ca^{2+} levels were higher in the menopausal women than during all phases of the menstrual cycle in the younger women.

Magnesium can be measured in the blood in four forms: total Mg, protein-bound Mg, Mg complexed to small anion ligands, and the biologically active Mg^{2+} (8). The ionized fraction of Mg has been shown to be labile and to correlate with the levels of intracellular free Mg in the red blood cells of patients with several types of chronic cardiovascular disease (9).

The present study involved healthy young men and healthy young women of similar age. We compared their serum levels of cations with those of older women to explore the hypothesis that these active divalent cations and their ratios are important to cardiovascular health (4). Increases and decreases in levels of the sex hormones (particularly estrogen) have been implicated in various cardiovascular diseases (1).

MATERIALS AND METHODS

Subjects and Blood Samples

Twenty-five healthy young male volunteers (aged 24–35 years) were recruited for this study. Their mean body mass index was 25.3 ± 4 kg/m^2. The group included 16 white men, 6 black men, and 3 Asian men. The study was approved by our internal review board, and we obtained written consent from the volunteers. Nonfasting blood samples were drawn under anaerobic conditions in the midafternoon. The serum was separated from the cells and stored at −10° to −20°C for cation analysis and at −79°C for hormone assays. None of the volunteers were taking any medications.

We also studied 25 menopausal women and 15 young women similar in age to the young men. The young women were studied at five different stages of the menstrual cycle, as described previously (5). The data for these two groups have been reported elsewhere (7).

Hormone and Cation Assays

Estradiol, progesterone, and testosterone levels were determined using Coat-a-Count RIA kits (Diagnostic Products Corporation, Los Angeles, CA). Levels of Ca^{2+} and Mg^{2+} were measured with Nova Stat Profile 8 (NOVA Biomedical, Waltham, MA) ion-selective electrodes for Ca^{2+} and Mg^{2+}, respectively (8). Total Mg was measured with a Kodak DT 60 colorimetric instrument (Rochester, NY) that uses a formazan dye complex (8). The Ca^{2+}/Mg^{2+} ratios were calculated, as was the percentage of Mg^{2+}. The normal reference ranges for our laboratory are as follows: Ca^{2+}, 1.09–1.33 mM/L; Mg^{2+}, 0.54–0.64 mM/L; total Mg, 0.74–0.96 mM/L; and percentage of Mg^{2+}, 61%–75% (8).

Statistical Analysis

Where appropriate, the data are presented as means ± SD. The relations between two variables were analyzed by simple linear regression (with 95% confidence limits) using Microcal Origin software (Northampton, MA). Correlation analyses were accomplished with Pearson's two-tailed significance test. The unpaired Student's *t*-test was used to ascertain relations between two groups. $P<.05$ was considered statistically significant.

TABLE 1

Serum hormone levels of normally cycling women, postmenopausal women, and healthy men.

Group	Mean (±SD) level of indicated hormone: Estrogen (pg/mL)	Progesterone (ng/mL)	Testosterone (ng/dL)
Normally cycling women*			
Follicular phase	41.05 ± 13.15	0.68 ± 0.34	18.55 ± 8.15
Ovulatory phase	184.19 ± 3.78	3.31 ± 5.65	26.97 ± 14.21
Postmenopausal women*	16.48 ± 9.45	0.26 ± 0.18	17.90 ± 9.48
Healthy men	44.89 ± 13.00	0.78 ± 0.28	470.78 ± 159.48

* Data are means (±SD) and are taken from reference no. 7 with permission.

Muneyyirci-Delale. Serum ionized magnesium. Fertil Steril 1999.

RESULTS

The mean (±SD) age of the male volunteers was 27.7 ± 2.87 years (range, 24–35 years). They were healthy, nonsmoking postdoctoral colleagues and residents. Table 1 shows the mean serum levels of estrogen, progesterone, and testosterone for all the subjects.

When we compared these levels with those of age-matched young women and postmenopausal women, we found that the serum estrogen levels of the men were in the same range as those of the young women in the early follicular (low serum estrogen) stage of the menstrual cycle and significantly higher than those of the postmenopausal women. The testosterone levels of the men were 20- to 30-fold higher than those of either group of women. The serum progesterone levels of the men were similar to those of the young women in the follicular stage, as reported previously (10), and significantly higher ($P<.001$) than

TABLE 2

Serum cation levels of normally cycling women, postmenopausal women, and healthy men.

	Mean (±SD) level of indicated parameter			
	Normally cycling women*			
Cation	Follicular phase	Ovulatory phase	Postmenopausal women*	Healthy men
Mg^{2+} (mM/L)	0.55 ± 0.04	0.53 ± 0.04	0.56 ± 0.04	0.55 ± 0.04
Ca^{2+} (mM/L)	1.19 ± 0.04	1.20 ± 0.05	1.24 ± 0.04	1.18 ± 0.03
Total Mg (mM/L)	0.83 ± 0.07	0.80 ± 0.07	0.83 ± 0.08	0.80 ± 0.08
Ca^{2+}/Mg^{2+} ratio	2.17 ± 0.18	2.29 ± 0.20	2.24 ± 0.18	2.18 ± 0.18
Percentage of Mg^{2+}	66.67 ± 3.98	66.33 ± 4.30	67.24 ± 4.89	68.68 ± 4.35

* Data are means (±SD) and are taken from reference no. 7 with permission.

Muneyyirci-Delale. Serum ionized magnesium. Fertil Steril 1999.

those of the menopausal women. The mean estrogen, testosterone, and progesterone levels in the young men, when normalized for body weight or body mass index (e.g., either <25 or >25), did not differ significantly ($P>.05$).

Table 2 shows the mean (±SD) serum levels of Mg^{2+}, Ca^{2+}, and the total Mg, the Ca^{2+}/Mg^{2+} ratios, and the percentages of Mg^{2+} for the men in blood samples obtained at the same time as those used for the hormonal analyses. The serum levels of Mg^{2+} and total Mg in the young men were similar to those in the cycling women at the follicular (low estrogen and low progesterone) stage (5) and similar to those in the menopausal women. However, the levels of total Mg tended to be lower than those in the menopausal women (7).

It is of interest that the mean levels of Mg^{2+} in the men were not significantly higher than those during the high estrogen stage in the young women or in the menopausal women (7). The levels of Ca^{2+} in the men (range and mean) were similar to those in the young women at the follicular stage of the menstrual cycle and not significantly different from those at the ovulatory stage. The levels of Ca^{2+}, however, were lower than those of the menopausal women; the difference was highly statistically significant ($P<.0001$).

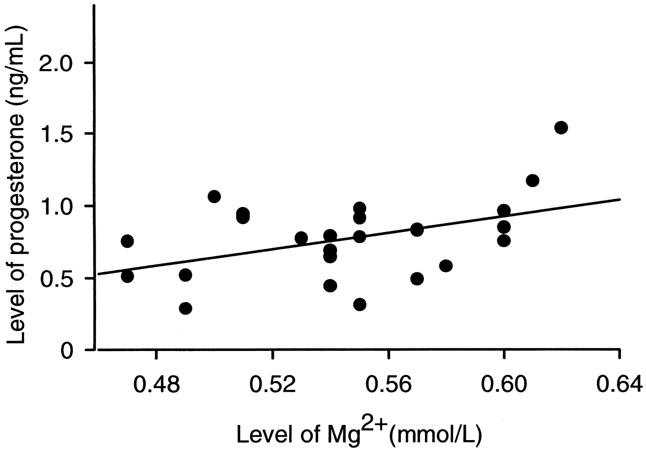
Given that the levels of Mg^{2+} and total Mg in the menopausal women were inversely related to the concentrations of estrogen (7), it was surprising that the levels of Mg^{2+} in the men were directly and significantly related to the levels of progesterone ($P<.05$) (Fig. 1). In addition, the Ca^{2+}/Mg^{2+} ratios were inversely related to the progesterone concentrations ($P<.05$) (Fig. 2). These significant relations between progesterone and Mg^{2+} levels as well as the Ca^{2+}/Mg^{2+} ratios did not seem to be affected by the racial composition of our population, at least with respect to whites and blacks. The number of Asians (n = 3) was too small to use for a statistically valid pool. We found no relations or correlations ($P>.05$) between levels of the cations and testosterone or estrogen or between levels of the sex steroids and total Mg in young men, either at face value or normalized for body mass index.

DISCUSSION

The present study complements our previous work, in that a group of healthy young men was found to have serum concentrations of the divalent cations similar to those of young women during the low estrogen and progesterone phase of the menstrual cycle. The mean estrogen levels of the men also were similar to those of the young women during that phase. However, their Mg^{2+} levels also were

FIGURE 1

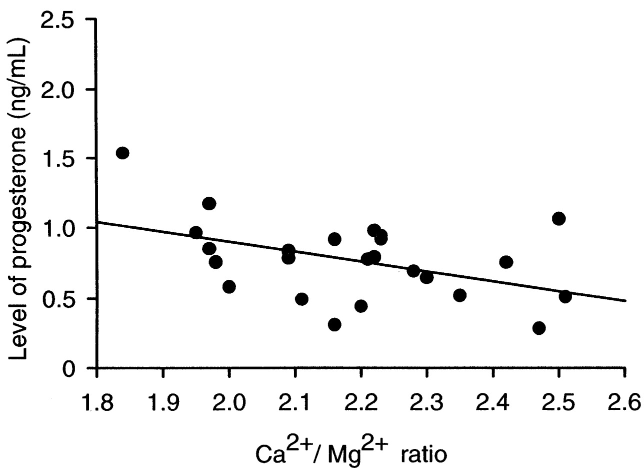
Relation of serum progesterone level to serum Mg^{2+} level in young men. $r = 0.44$. $P<.05$ (progesterone level versus Mg^{2+} level.)



Muneyyirci-Delale. Serum ionized magnesium. Fertil Steril 1999.

FIGURE 2

Relation of serum progesterone level to serum Ca^{2+}/Mg^{2+} ratio in young men. $r = 0.45$. $P<.05$.



Muneyyirci-Delale. Serum ionized magnesium. Fertil Steril 1999.

similar to those of the menopausal women, whose estrogen levels on the average were much lower than those of either the young men or the young women during the follicular phase, and whose Mg^{2+} levels were significantly and inversely related to their serum estrogen levels. The mean Ca^{2+} level of the young men was significantly lower than that of the menopausal women, who are known to have a higher rate of bone metabolism (3).

With respect to progesterone levels, Zumoff et al. (10) reported a mean (±SD) serum level of progesterone (0.214 ± 0.05 ng/mL) in healthy older men that is approximately one-fourth the level we demonstrated in healthy young men (mean [±SD] age, 44 ± 7.1 years; range, 37–51 years). Zumoff et al. drew blood samples from 7 older men early in the morning (6 A.M. to 9 A.M.) and pooled them. We drew blood samples from 25 young men in the midafternoon and analyzed them separately. However, the reference range for progesterone levels in adult men is 0.13–0.97 ng/mL (11), which clearly encompasses the levels found in both groups of men. In addition, the progesterone levels we found in the young men are similar to those in young women during the follicular phase of the menstrual cycle, as Zumoff et al. (10) also reported.

Estrogen has been cited as the primary protector of the cardiovascular system in different studies, with many examples in the literature suggesting that it is estrogen that protects the cardiovascular system in young women (12–14). In a recent Letter to the Editor, Simpson and Davis (15) provided support for the idea that estrogen protects the cardiovascular system in men also. They pointed out that testosterone may be "a circulating precursor for the local synthesis of estrogen in target [cardiovascular] organs" (15).

To our knowledge, we are the first to demonstrate a significant relation between the serum level of Mg^{2+} (which we believe protects the cardiovascular system) (4) and the serum level of progesterone in young men. Given that the concentration in young men is similar to that in young women during the follicular phase of the menstrual cycle, as reported previously (10), and higher than that in menopausal women, progesterone may have some heretofore unrecognized influence on the cardiovascular health of men. Some experimental work already has provided some support for this idea. For example, progesterone was found to inhibit arterial smooth muscle cell proliferation and cardiac fibroblast growth (16, 17) and to induce endothelium-independent relaxation of rabbit coronary arteries (18, 19), similar to what has been reported for estrogen.

However, there is some controversy in the literature regarding the endothelium-dependent dilation of blood vessels by the two hormones. Some studies show that progesterone opposes the flow-mediated vasodilatory effects of estrogen, whereas others do not (20, 21). It should be noted that concentrations of hormones that are biologically active in in vitro cellular and tissue studies are much higher than those found circulating in the blood. It is known that the two concentrations are not comparable (22).

Celemajer et al. (23) assessed endothelial function in connection with aging in men and women. They showed that flow-mediated dilation (which is endothelium-dependent) declined in men >40 years of age but was stable in women until approximately 50 years of age, when a steep decline occurred (23). They concluded that estrogen protects the systemic arteries, particularly the endothelial cells. Our findings suggest the need for careful reexamination of the effects of progesterone on the endothelial cells of blood vessels. Because it has been demonstrated that progesterone levels do not change significantly with age in men (24), it should be interesting to study whether a low progesterone level is a marker for cardiovascular disease.

Progesterone has been reported to have a direct action on circulating noradrenaline when administered to men. It reduces noradrenaline levels and thereby reduces sympathetic tone (25). Progesterone also is increased by stress reactions in men, in relation to activation of the pituitary-adrenal-cortical axis (26). Both these effects involve hormonal secretions that are intimately related to the availability of free divalent cations. Whether these are protective events remains to be determined, but the possibility seems plausible.

Another effect of progesterone that may be cardioprotective and that also involves cations is its ability to reduce a stimulated rise in intracellular Ca^{2+} in platelets and therefore possibly modulate atherosclerosis (27). An unrelated effect of progesterone on Ca^{2+} that should be mentioned for the sake of completeness is its known ability to trigger an increase in Ca^{2+} influx into sperm cells, stimulating the acrosome reaction that is necessary for fertilization. This effect is specific for this tissue (28), however, and occurs when levels of the hormone are greatly elevated.

Some consideration probably should be given to the tissue origins of the sex steroids. The adrenal cortex is the predominant organ in men that synthesizes and secretes progesterone (11). In contrast, the testis contributes significantly, if not predominantly, to their levels of estrogen and testosterone (11). In young women, progesterone arises mainly from the corpus luteum in the ovary, whereas after menopause, this hormone comes from the adrenals (11). In both sexes, there is peripheral conversion of adrenal, ovarian, and testicular androgens to estrogen and testosterone (11). Finally, progesterone is an intermediate molecule in the synthesis of all steroid hormones from cholesterol (11).

It is thus possible that the link between circulating divalent cations and progesterone reflects enzymatic or secretory effects of these mineral ions in adrenal and other tissues or an effect of progesterone on the concentrations of these ions. It also may have a genetic origin. In other words, the question is whether Mg^{2+} and/or the Ca^{2+}/Mg^{2+} ratio regulates circulating progesterone in humans, irrespective of whether the adrenal gland is the target organ, or whether progesterone contributes to the regulation of circulating Mg^{2+}. None of these intriguing possibilities can be substantiated without further study, especially at the cellular level.

What can be said is that body mass index and body weight were not confounding factors in the present study. In addition, it should be mentioned that hormonal control mechanisms for circulating Mg^{2+} are beginning to be theorized about and described (29, 30). Whether progesterone in men is another such hormone should be considered and studied.

The comparatively low Ca^{2+} levels found in the young men in this study, on the average, bodes well for their cardiovascular health, because they may indicate a decreased tendency toward constrictive events and high blood pressure. The broad range of normal Mg^{2+} levels that we observed in young men should be noted and is similar to what we demonstrated previously in healthy young men (31). We recently described several patients with cardiovascular disease who had low Mg^{2+} levels and high Ca^{2+}/Mg^{2+} ratios in their circulating blood (4, 9, 32, 33). It may be important to monitor young men whose levels fall at the lower end of the scale because they may be more likely to have cardiovascular disease as they age. If our predictions hold true, these levels would be an easy marker to follow and a definitive prognostic sign for early intervention to prevent or at least ameliorate the outcome of cardiovascular disease. It is intriguing to conclude from our work that the metabolism of Mg is controlled differently in men and women, with estrogen predominating in women and progesterone in men.

A note of caution is required with respect to race and dietary habits. It recently has been demonstrated that race can have a significant effect on circulating levels of Mg^{2+} (34). Our population sample was too small to assess these factors. It also has been shown that short-term changes in dietary intake of total Mg can alter serum levels of Mg^{2+} (31). However, the amounts supplemented in the diet were large and would not be present in a normal, everyday diet.

In conclusion, our study indicates that serum levels of Mg^{2+} in young men are directly and significantly related to serum levels of progesterone and that the Ca^{2+}/Mg^{2+} ratio is inversely related to serum levels of progesterone. The data are compatible with (but do not prove) the possibility that serum Mg^{2+} and/or the Ca^{2+}/Mg^{2+} ratio contribute to the regulation of circulating progesterone in humans or that progesterone contributes to the regulation of circulating Mg^{2+}.

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