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The Association Between Calcium, Magnesium, and Ratio of Calcium/Magnesium in Seminal Plasma and Sperm Quality

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Abstract The study aimed to examine the relationships between calcium, magnesium, and calcium/magnesium ratio in semen plasma and sperm quality. It was a cross-sectional study based on a program aiming at promoting the reproductive health in less-developed areas. A total of 515 men aged between 18 and 55 years provided semen specimens at family planning clinics in Sandu County, Guizhou Province, China. Total calcium and magnesium concentrations in semen plasma were measured with flame atomic absorption spectrometry. Sperm quality, including sperm motility and concentration, was evaluated by using a computer-assisted sperm analysis method. The medians of seminal plasma calcium, magnesium, and zinc concentrations were 9.61, 4.41, and 2.23 mmol/l, respectively. Calcium concentration and calcium/magnesium ratio were negatively associated with sperm concentrations ($\beta = -0.47$, $P = 0.0123$ for calcium; $\beta = -0.25$, $P = 0.0393$ for calcium/magnesium ratio) after adjusting for zinc and other covariates. In stratified analyses, the association between calcium and sperm concentrations only persisted among subjects with a calcium/magnesium ratio of ≤ 2.5 ($\beta = -0.71$, $P = 0.0268$). In the same stratum, magnesium was associated with increased sperm concentration ($\beta = 0.73$, $P = 0.0386$). Among subjects with a calcium/magnesium ratio of >2.5, neither calcium nor magnesium was associated with sperm concentration. In conclusion, total calcium and magnesium concentrations were associated with sperm concentration among subjects with a lower calcium/magnesium ratio. The calcium and magnesium ratio had a modifying effect on the associations of calcium and magnesium with sperm concentration.

Keywords Sperm quality · Calcium · Magnesium · Calcium/magnesium ratio

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Introduction

Human seminal plasma contains high concentrations of elements, like calcium, magnesium, and zinc in bound and ionic forms. These elements are important for the maintenance of normal sperm physiology including spermatogenesis, maturation, acrosome reaction, and fertilization [1, 2]. However, the role of seminal concentrations of calcium and magnesium on conventional sperm quality is not fully understood. Findings from previous studies have been conflicting. Lower concentrations of ionized calcium were reported to be associated with decreased sperm motility [3–5], while other studies showed that there was no association between total calcium levels and motility [6–9]. A significant positive correlation between magnesium level and sperm concentration [9] was reported, whereas other studies did not observe any association between magnesium level and sperm quality [8, 10].

Calcium and magnesium belong to the same family in the periodic table. They have similar homeostatic regulatory systems and can potentially antagonize each other in many physiological activities [11, 12]. Recent studies reported that the dietary calcium/magnesium intake ratio had significant modifying effects on the associations between intakes of calcium and magnesium and risk of cancer [12], mortality due to cardiovascular disease, total mortality [12], and colorectal neoplasia risk or recurrence [13, 14]. These studies indicated that calcium and magnesium balance may have an important modifying effect on the function of calcium and magnesium on disease outcome. Furthermore, a high serum calcium/magnesium ratio and low serum magnesium levels were significantly associated with prostate cancer and the association for the ratio was independent of serum magnesium or calcium levels [15], suggesting that the calcium/magnesium ratio is critical for prostate or other male physiological functions.

Thus, not considering, the balance of calcium and magnesium may contribute to the inconsistent results on the associations between calcium and magnesium and sperm quality. In this study, we examined the associations between calcium, magnesium, and the ratio of calcium/magnesium in seminal plasma and sperm quality by using data from a population-based study.

Materials and Methods

Study Population

The cross-sectional study was based on a primary health program that aimed at promoting reproductive health in less-developed areas, which provided free medical counseling on reproductive health as well as free semen quality assay for couples of reproductive age. The study was conducted from July to August 2012 in Sandu County (Autonomous County of Shui nationality), Guizhou Province, China. Men aged between 18 and 55 years were identified and invited to participate in the study by local service providers.

A total of 1046 men (approximately 75 % of those who were approached) agreed to participate. Among them, 790 men provided semen samples. Men who had testicle problems including cryptorchidism and testicular volume <15 ml ($n = 99$) or epididymis problems ($n = 79$) were excluded from the present study, as well as those who had spermaduct problems ($n = 10$) or varicocele ($n = 13$). Among the remaining 589 men, 66 subjects without measurements of calcium, magnesium, and zinc levels were further excluded from the present study since (1) their semen volume was insufficient for element assay, (2) seminal plasma could not be separated from their semen sample, or (3) samples were lost due to transportation damage and experiment error. Eight subjects were excluded due to missing information on birth date. A total of 515 subjects (49.2 %) were finally included in the present analysis.

The study was approved by the Committees for Protection of Human Subjects at the Shanghai Institute of Planned Parenthood Research. All the participants in the study gave written informed consent.

Data and Bio-Sample Collections

Information was obtained on demographics, lifestyle factors (smoking, alcohol and caffeine consumption), history of chemical substance contact, and history of infertility through an in-person interview.

A single semen sample was collected from each man after an abstinence period of 2 to 7 days. Semen samples were collected by masturbation into 25-ml sterile polystyrene jars and were analyzed within 1 h of ejaculation. An aliquot of semen was centrifuged at 3000 rpm for 5 min. Then, the supernatant was frozen without preservatives and stored at −20 °C temporarily. All the samples were shipped to the laboratory at the Shanghai Institute of Planned Parenthood Research (Shanghai, China) on dry ice and stored at −80 °C for the assays of calcium, magnesium, and zinc concentrations. In this pilot investigation, we collected only one semen sample (with minimum volume being 0.5 ml of fluid) to obtain a higher participation rate, because few men are willing to donor their semen samples repeatedly.

Semen Analysis

Semen analysis is widely used for testing male reproductive system diseases and is the cornerstone for testing reproductive functions of the male partners among subfertile couples. Semen volume, sperm concentration, motility, and morphology are the commonly used parameters to describe semen quality. World Health Organization (WHO) has produced and updated the semen analysis manual to provide methods on how to perform semen analysis and the criteria for normal levels of semen parameters.

In the study, semen analyses to obtain sperm concentration and motility were conducted after liquefaction at the clinics, in accordance with the WHO guidelines [16]. Computer-assisted sperm analysis (CASA) (WLJY-9000, Beijing, People's Republic of China) was used to evaluate sperm concentration and motility. Motility and forward motility were defined as the percentage of total motile sperm and the percentage of progressively motile sperm, respectively. The same professional technician performed the semen analysis for all specimens to ensure consistency. The following parameters on motility trajectory were also derived from the analyses: straight line velocity (VSL), linearity (LIN), curvilinear velocity (VCL), average path velocity (VAP), straightness (STR), amplitude of lateral head displacement (ALH), beat-cross frequency (BCF; Hz), and wobble (WOB).

Assay of Zinc, Calcium, and Magnesium in Semen Plasma

Total zinc, calcium, and magnesium concentrations in semen plasma were measured by using flame atomic absorption spectrometry (FAAS) by a BH5500S device (Bohui Innovation Technology Co., Ltd., Beijing, China). An aliquot of semen plasma was centrifuged at 5000 rpm for 10 min, and 100 μl of the supernatant was drawn and mixed into a 100-μl solution containing 10 % dilute nitric acid. Then, 10 μl of the mixture was drawn and mixed into a 1.2-ml solution called Bohui multi-element examination reagent (specified for BH5500S device on element measurement) and prepared for the assay of calcium, magnesium, and zinc. The calibration solution in the determinations was provided by the Bohui Company, and accuracy of the calibration solution was set according to the national standard material for zinc (GBW08620), for calcium (GBW(E)080118), and for magnesium (GBW(E)080126). The detection limits were 0.025 mmol/l for calcium, 0.021 mmol/l for magnesium, and 0.002 mmol/l for zinc, respectively.

Statistical Analysis

SAS version 9.2 (SAS Institute Inc., Cary, North Carolina, USA) was used to conduct all statistical analyses. Spearman's rank correlation coefficients were used to explore the intercorrelation between calcium, magnesium, zinc, and calcium/magnesium ratio. In linear regression analyses, calcium, magnesium, and zinc concentrations and calcium/magnesium ratio were transformed by using the natural log (ln) due to the skewed distribution, as well as sperm concentration. Multiple linear regression analysis was conducted to examine the associations between concentrations of calcium, magnesium, or calcium/magnesium ratio, and semen parameters (including concentration, motility, and sperm parameters on motility trajectory), after controlling for covariates that may be associated with semen parameters. Two adjusted models were used. In the first model, only zinc concentration was adjusted, since zinc was shown to be a strong predictor of sperm parameters in previous studies [17, 18]. In the second model, the following covariates were further included in the multiple linear regression analysis: age, body mass index (BMI; <18.5, 18.5–<25, and ≥25 kg/m^2), smoking (current smoker vs. not current smoker), alcohol drinking (current drinker vs. not current drinker), race/ethnicity (Shui, Bouyei, Miao, and other nationalities), history of chemical substance contact (yes vs. no), and abstinence time. When analyzing the effect of calcium or magnesium, further adjustments were made for magnesium or calcium in the model. BMI was calculated as weight (kg) divided by the square of height (m^2).

Stratified analyses were performed according to calcium/magnesium ratio to examine the associations of calcium and magnesium with semen parameters. Considering that a history of infertility may influence the association between calcium and magnesium and semen parameters, a sensitive analysis was performed by excluding 146 men with a history of infertility. Men with a history of infertility were older than those without. However, other characteristics, including BMI, race, education, smoking, drinking, and history of chemical contact, were similar between men with and without a history of infertility. Infertility was defined by WHO as the inability to achieve a clinical pregnancy after 12 months or more of regular unprotected sex intercourse [19].

Among 515 subjects, 20 (3.9 %) had a history of urogenital system disease (urinary tract infection, prostatitis, or kidney stone); 23 (4.5 %) reported a history of other diseases (one with hypertension; five with pulmonary tuberculosis; nine with hepatitis, cholecystitis, or gallstone; seven with gastroenteritis, duodenal ulcer, or gastrorrhagia; one with epilepsy). On investigation, nine subjects were on medication. To exclude the effects of disease history and medication use on the association, the abovementioned analyses (n = 473) were repeated among those without disease history and medication use.

Results

The medians (25th–75th percentiles) of sperm concentration, total motility, and forward motility among the semen samples were 44.9 × 10^6/ml (22.0–76.4 × 10^6/ml), 56.0 % (38.0–73.0 %), and 42.9 % (28.0–54.0 %), respectively. According to WHO reference limits for semen parameters on normal fertility (sperm concentration ≥15 × 10^6/ml, the proportion of motile sperm ≥40 %, and the proportion of forward motile sperm ≥32 %) in fifth edition [16], 14 % of the samples were less than the reference limits in sperm concentration, 26.6 % in total motility, and 29.9 % in forward motility.

Table 1 shows the characteristics of the study population and the corresponding semen parameters, including sperm concentration, forward motility, and motility. More than 60 % of the participants were younger than 35 years of age. A majority of the subjects are of the Shui ethnic group (56.7 %), followed by Bouyei (21.6 %), Miao (15.2 %), and other ethnic groups (6.4 %). Nearly half the participants (47.3 %) had a junior high school of education, followed by primary school (37.4 %) and senior high school (15.3 %). More than half of the participants were current smokers (57.1 %) or current drinkers (57.8 %), and 48 participants

Table 1 Characteristics of the study population and sperm quality

	N	Sperm concentration ($\times 10^6$/ml)[a]	Forward motility (%)[a]	Motility (%)[a]
Age (years)				
<30	202	44.9 (24.6–74.8)	47.4 (33–57.3)	61.3 (43.6–75)
30–34	129	38 (18–72)	40 (25–51)	54 (35–71)
35–39	111	47.4 (22–96)	41 (25–51)	54 (36–68)
≥40	73	53.6 (31–73.8)	38.8 (22.6–54)	48.6 (33.9–75)
Ethnicity				
Shui ethnic group	291	47 (24.9–84)	43 (28–54.8)	57 (37.5–74)
Bouyei ethnic group	111	42.7 (18.4–68.8)	43 (28.6–53.9)	54 (39–72)
Miao ethnic group	78	39.8 (19–62)	43 (30–54)	54 (42.8–73)
Other ethnic groups	33	44.9 (32–72.8)	40 (29–50)	57 (37.5–74)
Education				
Primary school	191	47 (21–79.3)	41.4 (23.6–51.0)	53.2 (35.3–72)
Junior high school	241	43.1 (26–74.8)	42.9 (31.2–55.9)	57 (40.1–72.9)
Senior high school	78	39.5 (16.6–71.4)	44.4 (25.1–58)	56.8 (34–77.9)
Current smoker				
Yes	290	44 (24.9–74.8)	43 (29–54)	57.1 (40.8–72.9)
No	218	44.5 (19–79.1)	41.2 (25–53.9)	53.7 (35–74)
Current drinker				
Yes	295	45.4 (27–74.4)	43.4 (28.9–54.1)	58.4 (41–72.9)
No	215	42 (18.4–81.3)	41 (25.3–54)	53 (36.9–73.8)
History of chemical substance contact				
Yes	48	33.6 (17.8–71.6)	37.2 (24.3–49)	48.2 (33–66.3)
No	448	46.1 (24.6–76.3)	43.1 (29.0–54.0)	57 (39.5–74)
Body mass index (kg/m^2)				
<18.5	32	46.5 (31.3–68.9)	46.3 (34.9–60.4)	57.0 (47.2–72.5)
18.5–25	375	44 (21–78.1)	42.9 (28–54)	57 (36.9–74)
≥25	107	45 (24.7–77)	42 (24.4–53.4)	53 (38–71)

[a] Median (25th–75th percentiles)

(9.7 %) reported a history of chemical substance contact. There was a decreased trend of sperm motility with increasing age. Moreover, the subjects with a history of contact with chemical substances tended to have decreased sperm concentration, forward motility, and motility (Table 1).

The medians of calcium, magnesium, and zinc concentrations in seminal plasma were 9.61, 4.41, and 2.23 mmol/l, respectively. The median of the calcium/magnesium ratio was 2.46 with an interquartile range from 1.86 to 3.23 (Table 2). There were significant positive intercorrelations among calcium, magnesium, and zinc with Spearman's rank correlation coefficient (r) up to 0.87. While the calcium/magnesium ratio was negatively correlated with zinc ($r = -0.64$, $P < 0.0001$) and calcium ($r = -0.41$, $P < 0.0001$), strong negative correlation was observed between the calcium/magnesium ratio and magnesium ($r = -0.83$, $P < 0.0001$) (Table 3).

Table 4 presents the associations between calcium, magnesium, and the calcium/magnesium ratio in seminal plasma and sperm concentration, forward motility, and motility. Both calcium and magnesium concentrations were positively associated with sperm concentration. However, after adjusting for zinc concentration, the association of calcium turned negative ($\beta = -0.42$, $P = 0.0128$), while the association of magnesium was still positive, but no longer statistically significant. After further adjusting for age, BMI, smoking, alcohol drinking, race/ethnicity, history of contact with chemical substance, and abstinence time, the association did not essentially change (calcium: $\beta = -0.47$, $P = 0.0123$, magnesium: $\beta = 0.20$, $P = 0.1203$). There was a significantly negative association between the calcium/magnesium ratio and sperm concentration. After adjusting for zinc and the covariates noted above, the association persisted with an attenuated regression coefficient ($\beta = -0.25$, $P = 0.0393$). On the other hand, both cations and the calcium/magnesium ratio were not associated with sperm motility and forward motility after adjusting for the covariates noted earlier.

To explore the impact of the calcium/magnesium ratio on the associations of calcium and magnesium with sperm concentration, stratified analyses were performed according to the

Table 2 Concentrations of calcium, magnesium, and zinc and calcium/magnesium ratio in seminal plasma

	X (±SD)[a]	Median	Interquartile range (25th–75th percentiles)	Minimum-maximum
Zinc (mmol/l)	2.38 (1.36)	2.23	1.42–3.16	0.26–6.52
Calcium (mmol/l)	9.61 (3.76)	8.99	6.87–11.68	0.91–25.04
Magnesium(mmol/l)	4.41 (2.80)	3.71	2.33–5.93	0.41–15.37
Calcium/magnesium ratio	2.74 (1.24)	2.46	1.86–3.23	0.69–11.29

[a] Mean (±standard deviation)

median value (2.5) of calcium/magnesium ratio (Table 5). In the stratum where the calcium/magnesium ratio was ≤2.5, the positive association between calcium and sperm concentrations persisted, with the regression coefficient slightly increased ($\beta = -0.71$, $P = 0.0268$). In addition, the association between magnesium and sperm concentration became statistically significant ($\beta = 0.73$, $P = 0.0386$). The associations of calcium and magnesium with sperm concentration disappeared among subjects with a calcium/magnesium ratio of >2.5.

There was no statistically significant association between calcium, magnesium, calcium/magnesium ratio and sperm motility trajectory parameters after adjusting for the covariates noted earlier (data not shown).

When the analyses were restricted to subjects without infertility history, the results did not essentially change, but the regression coefficients increased slightly (data not shown). Analyses were further restricted to those without a history of systemic disease and medication use, and the estimates were found to have changed slightly (data not shown).

Discussion

In this study, calcium level in seminal plasma was observed to be associated with decreased sperm concentration, but not motility, after adjusting for zinc and magnesium concentrations, particularly among the subjects with a lower calcium/magnesium ratio (≤2.5). Calcium plays an important role in sperm physiology including motility, capacitation, and acrosome reaction [1]. However, the role of seminal calcium in sperm quality has not yet been elucidated. In studies where total calcium was measured, there was no association between calcium concentration and sperm motility [3, 4, 8, 9]. However, the results on ionized calcium and sperm motility were inconsistent. Several studies [3–5] showed that ionized calcium was positively associated with sperm motility, while Magus [6] observed that there was no association of ionized calcium with the proportion of progressively motile sperm. In Schmid's study [20], a negative association was noted between sperm calcium and motility. In the current study, no association was found between total calcium concentration and sperm motility. Our findings are in agreement with those of previous studies [3, 4, 8, 9] where total calcium was measured.

Table 3 Spearman's rank correlation coefficients between calcium, magnesium, and zinc concentrations and calcium/magnesium ratio in seminal plasma

	Calcium	Magnesium	Calcium/magnesium ratio
Zinc	0.82*	0.87*	−0.64*
Calcium		0.83*	−0.41*
Magnesium			−0.83*

*$P < 0.0001$

In the current study, calcium was negatively associated with sperm concentration, which was inconsistent with two previous studies. However, Sorensen's study [8] recruited only 50 healthy men to analyze the impact of calcium on semen parameters, while another study [9] used a correlation coefficient to describe the effect of calcium without adjusting for zinc or other potential confounders. In the current study, the positive association between calcium and sperm concentrations became negative after adjusting for zinc.

Magnesium, with a high concentration in semen, plays a very important role in cell physiology, especially in enzymatic systems. Magnesium is involved as a cofactor in many enzymatic reactions including nucleic acid synthesis and energy metabolism [21]. In the current study, magnesium was positively associated with sperm concentration among the subjects with a lower calcium/magnesium ratio (≤2.5). Not considering calcium/magnesium ratio in previous studies may explain the inconsistency of results between this study and previous studies [8–10]. There was no association between magnesium and sperm motility in the current study, which was in line with previous studies [8–10].

In the current study, the associations between calcium and magnesium and sperm concentration existed only among subjects with a lower calcium/magnesium ratio. This suggested that the calcium and magnesium balance may have a modifying effect on the associations of calcium and magnesium with sperm parameters. In addition, the calcium/magnesium ratio was negatively associated with sperm concentrations. This suggested that the

Table 4 Associations between calcium, magnesium, and calcium/magnesium ratio in seminal plasma and sperm quality

	Crude model[a]		Adjusted model 1[b]		Adjusted model 2[c]	
	β	*P*	*β*	*P*	*β*	*P*
Calcium						
Sperm concentration	0.31	0.0018	*−0.42*	*0.0128*	*−0.47*	*0.0123*
Forward motility	1.28	0.5425	1.22	0.7334	−0.08	0.9841
Motility	5.11	0.0549	−0.44	0.9230	−2.07	0.6836
Magnesium						
Sperm concentration	0.31	<0.0001	0.10	0.3996	0.20	0.1203
Forward motility	0.95	0.4406	1.62	0.5071	2.70	0.3246
Motility	3.94	0.0115	2.55	0.4084	3.91	0.2563
Calcium/magnesium ratio						
Sperm concentration	−0.53	<0.0001	*−0.32*	*0.0079*	*−0.25*	*0.0393*
Forward motility	−1.33	0.5046	−1.15	0.6530	−2.18	0.4081
Motility	−5.67	0.0240	−3.00	0.3512	−3.54	0.2836

Only the italicized data shown the statistical significance in the adjusted models

[a] Crude model: no risk factor was adjusted

[b] Adjusted model 1: zinc concentration was adjusted

[c] Adjusted model 2: In addition to zinc, age, BMI, nationality, smoking, drinking, chemical substance contact history, and abstinence time were further adjusted. When analyzing the associations between calcium and sperm quality, magnesium was also adjusted to assess whether the associations were independent of magnesium and vice versa

calcium/magnesium ratio may also have an independent effect on sperm quality. Although these findings are novel, they are biologically plausible. Magnesium and calcium antagonize each other in many physiological activities, such as oxidative stress [22], DNA repair, cell differentiation and proliferation, apoptosis, and angiogenesis [11, 23], which may also be involved in the development of spermatogenesis and sperm maturation. In addition,

Table 5 Associations between calcium and magnesium in seminal plasma and sperm quality after stratification stratified by calcium/magnesium ratio

	Crude model[a]		Adjusted model 1[b]		Adjusted model 2[c]	
	β	*P*	*β*	*P*	*β*	*P*
Calcium/magnesium ≤2.5						
Calcium	0.22	0.1136	−0.47	0.0908	*−0.71*	*0.0268*
Magnesium	0.37	0.0013	0.28	0.3117	*0.73*	*0.0386*
Calcium/magnesium >2.5						
Calcium	0.16	0.3040	−0.38	0.1179	−0.49	0.1463
Magnesium	0.14	0.1938	−0.14	0.3508	0.11	0.6130

Only the italicized data shown the statistical significance in the adjusted models

[a] Crude model: no risk factor was adjusted

[b] Adjusted model 1: zinc concentration was adjusted

[c] Adjusted model2: in addition to zinc, age, BMI, nationality, smoking, drinking, chemical substance contact history, and abstinence time were further adjusted. When analyzing the associations between calcium and sperm quality, magnesium was also adjusted to assess whether the associations were independent of magnesium and vice versa

several recent studies reported that the dietary calcium/magnesium intake ratio had modifying effects on the effects of intakes of calcium and magnesium on risks of mortality [12] and cancer [12–14]. Moreover, a high serum calcium/magnesium ratio itself was significantly associated with the risk of high-grade prostate cancer [15]. Further studies are needed to corroborate these findings and explore the mechanism.

Although previous studies have explored the relationships of calcium or magnesium alone with sperm quality, the current study was the first to examine the association between the calcium/magnesium ratio and sperm quality in detail. The current study had a relatively larger sample size compared to that seen in earlier published studies. Furthermore, the current study adjusted for some potential confounders. However, several limitations need to be considered. First, as a cross-sectional study, the causal relationship between elements and sperm quality could not be inferred. Second, a higher proportion of subjects who volunteered to participate in the study had a history of infertility compared to the source population. However, the findings may not be solely explained by the selection bias since the results remained unchanged after those with a history of infertility were excluded. Additionally, all participants were unaware of their element levels at recruitment. Third, the generalization of the findings should be carefully considered since the study was conducted in one remote mountainous county of Guizhou Province, where the distribution of ethnicity is very unique.

Conclusion

In this pilot study of seminal plasma and sperm quality, total calcium concentration, magnesium concentration, and calcium to magnesium ratio in seminal plasma may affect sperm concentration, but not sperm motility. Moreover, the ratio of calcium to magnesium may in itself have a modifying effect on the associations of calcium and magnesium with sperm concentration.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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