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Association between calcium/magnesium ratio and insulin resistance in women with obesity and normal-weight women

Asociación entre la relación calcio/magnesio y la resistencia a la insulina en mujeres con obesidad y en mujeres con peso normal

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ABSTRACT

The aim of this study was to evaluate the association between the calcium/magnesium (Ca/Mg) ratio and insulin resistance in women with obesity and normal-weight women. This was a cross-sectional study with 128 women (62 women with obesity and 66 normal-weight women). We measured dietary minerals intake and analyzed magnesium and calcium biomarkers. Ca/Mg ratio in diet, plasma and urine were calculated. We have evaluated glycemic parameters. Women with obesity had low dietary magnesium, reduced plasma and erythrocyte magnesium concentrations, and elevated urinary magnesium excretion. Plasma calcium concentration was lower and urinary calcium excretion was higher in patients with obesity than in the normal-weight group. Dietary magnesium and calcium intake per kilogram of body weight per day was lower in obese women than in the control group. Ca/Mg ratio in plasma and urine were elevated in women with obesity. We found a significant correlation among magnesium biomarkers and calcium parameters. Ca/Mg ratio seems to be associated with insulin resistance in obese women.

Keywords: Calcium; Insulin resistance; Magnesium; Metabolism; Obesity.

RESUMEN

El objetivo de este estudio fue evaluar la asociación entre la relación Ca/Mg y la resistencia a la insulina en mujeres con obesidad y en mujeres con peso normal. El diseño del estudio fue transversal y participaron 128 mujeres (62 mujeres con obesidad y 66 mujeres con peso normal). Se analizó la ingesta de minerales en la dieta y se realizaron análisis de biomarcadores de magnesio y calcio. Se calculó la relación Ca/Mg en dieta, plasma y orina y se evaluaron los parámetros glicémicos. Las mujeres con obesidad tenían niveles bajos de magnesio en la dieta, concentraciones reducidas de magnesio en plasma y eritrocitos, y excreción urinaria de magnesio elevada. La concentración plasmática de calcio fue menor en pacientes con obesidad, y la excreción urinaria de calcio fue mayor que en el grupo de mujeres con peso normal. La ingesta dietética de magnesio y calcio por kilogramo de peso corporal por día fue menor en las mujeres con obesidad, que en el grupo control. La relación Ca/Mg en plasma y orina estaba elevada en mujeres con obesidad. Se encontró una correlación significativa entre los biomarcadores de magnesio y los parámetros de calcio. La relación Ca / Mg parece estar asociada con la resistencia a la insulina en mujeres con obesidad.

Palabras clave: calcio; Magnesio; Metabolismo; Obesidad; Resistencia a la insulina.

INTRODUCTION

Obesity is a chronic disease characterized by excessive fat accumulation that is the main contributing factor for the development of metabolic disorders in obese individuals. Adipose tissue accumulation, particularly intra-abdominal adipose tissue, is associated with increased secretion of proinflammatory cytokines, inducing thus the development of low-grade chronic inflammation and insulin resistance^{1,2}.

Researchers have studied mechanisms linking minerals to obesity-associated metabolic disorders. Magnesium is an important mineral in cellular energy metabolism, and regulation of insulin secretion and insulin action³. Magnesium is critical for the phosphorylation of the insulin receptor and other downstream signal kinases of the target cells because it is a necessary cofactor in all adenosine triphosphate (ATP) transfer reactions⁴. Calcium is essential for insulin secretion because calcium is crystallized with insulin during the granule maturation process, and the initial trigger for insulin granule fusion with the plasma membrane is a rise in intracellular calcium⁵. Thus, magnesium and calcium are critical for glycemic control.

However, individuals with obesity are magnesium-deficient⁶. Calcium regulates magnesium metabolism because calcium and magnesium compete for the same carriers in human renal cells apical membrane and in human intestinal brush border membrane^{7,8}. Therefore, excessive dietary intake of calcium may reduce intestinal absorption of magnesium, and excessive blood concentrations of calcium may reduce renal reabsorption of magnesium^{8,9}. Thereby, excess dietary and serum calcium may induce magnesium deficiency, impairing thus magnesium functions, for example improvement in insulin sensitivity^{8,9}.

Studies have shown the relevance of increased calcium/magnesium (Ca/Mg) ratio in the development of metabolic disorder^{10,11}. Evidence reviewed by Rosanoff¹² indicates that there was an increase in food Ca/Mg ratio from 1977 through 2007-8 for all American adult age-gender groups. The incidence of diagnosed type 2 diabetes mellitus increased sharply in the mid-1990s through 2008, the same timeframe in which the Ca/Mg intake ratio from foods for this population

went from largely below 3.0 to largely above 3.0¹³.

Population-based cohort studies conducted in China observed significant modifying effects of a Ca/Mg intake ratio, but not Mg or Ca intake alone, on the associations between intakes of Ca and Mg, respectively, with risk of total mortality and coronary heart diseases and possibly cancer¹⁰. A clinical trial involving 96 patients with hypertension showed that the ratio between serum calcium and magnesium concentrations is inversely associated with changes in insulin sensitivity index¹⁴. This fact suggests that the balance between calcium and magnesium concentrations in serum is relevant to protect against insulin resistance, an obesity-associated metabolic disorder.

Although there is consistent evidence in the published literature that magnesium and calcium play an important role on glycemic control, it is necessary to evaluate the influence of Ca/Mg ratio on insulin resistance because data are still scarce. Accordingly, the aim of this study was to evaluate the association between Ca/Mg ratio and insulin resistance in women with obesity and normal-weight women.

MATERIALS AND METHODS

This was a cross-sectional study involving 128 women. We classified participants in two groups: cases (women with obesity, $n = 62$) and controls (normal-weight women, $n = 66$). Patients who attended routine outpatient appointments at a clinic in Teresina city, Piauí State, Brazil were invited to participate. We recruited participants from March 2017 to February 2018 using convenience sampling.

Inclusion criteria were: women aged 20-50 years; body mass index (BMI) between 18.5 and 24.9 kg/m² (control group) or a BMI of 35 kg/m² or greater (obese women); non-smoker, non-pregnant and non-lactating woman; not be participating in another clinical study; not having diabetes mellitus, cancer, chronic renal failure, or inflammatory bowel diseases; and not using vitamin and mineral supplements and/or medicines that could affect the minerals levels and obesity-associate metabolic disorders. The control group included normal-weight women without any known disease and were used as a standard of comparison.

The Research Ethics Committee in Federal University of Piauí, Brazil approved this study (number 2.014.100). All participants signed informed consent form after clarification of our study, according to norms of National Research Council in Resolution 466/12¹⁵.

Nutritional status evaluation

Body weight (in kg to the nearest 0.1 kg) was measured with a reliable scale (Plenna®, Sao Paulo, Brazil) while participants were wearing light clothing and no shoes. Body height (in cm to the nearest 0.1 cm) was measured with a stadiometer (Seca®, Sao Paulo, Brazil). Position of the head was standardized by asking the subject to stand straight, heels together. Height and weight were measured three times, and the mean value of the three measurements was taken. All measurements were taken at a private clinical analysis laboratory¹⁶. Body mass index (BMI) was calculated as weight (kg)/ height (m)². We have used World Health Organization classification¹⁷. Participants reported their age. Waist circumference was measured using a flexible and inelastic tape, at the level of the umbilicus. Waist circumference cut-points as recommended by the World Health Organization¹⁸ were used as reference value.

Dietary magnesium and calcium intake evaluation

We used the three-day food record method to analyze dietary magnesium and calcium intake. Three-day food record covered two weekdays and one weekend day. We instructed respondents and presented them with examples on how to complete the food record. The amount of food consumed was determined with the use of photographs of dishes. All food records were checked by a dietitian. We have estimated dietary intake of these nutrients using the "NutWin" software, version 1.5 (Sao Paulo, Brazil)¹⁹. We used Estimated Average Requirement (EAR), in the Dietary Reference Intakes (DRIs), as a reference standard^{20,21}.

Reference values of dietary magnesium and calcium intake were: 255 mg Mg/d for women aged 19-30 y, and 265 mg Mg/d for women aged 31 y and older²⁰; and 800 mg Ca/d (women aged 19-50 y)²¹.

We imported dietary intake of magnesium and calcium into the Multiple Source Method (MSM) program, version 1.0.1 for adjustments of intrapersonal and interpersonal variability, corrected by statistical modelling techniques. We have estimated the usual dietary intake of magnesium and calcium with MSM using a logistic regression model. This program can be accessed through the MSM website (<https://nugo.dife.de/msm/>)^{22,23,24}.

Dietary intake data of magnesium and calcium were also energy-adjusted by the residual method, reducing the influence of differences in energy consumption on dietary magnesium and calcium intake. Dietary intake data of magnesium and calcium were energy-adjusted by nutrient calculation after verifying the normality of the data distribution^{25,26,27}.

Assessment of Biochemical Parameters of Magnesium and Calcium

Twelve milliliters of venous blood were collected in the morning period between 7 and 9 A.M. by a nurse at a private clinical analysis laboratory. Participants fasted for at least 12 hours before blood collection. Blood sample was distributed in three tubes: one containing sodium citrate anticoagulant to analyze magnesium and calcium (4 mL of blood), one blood tube without anticoagulant to analyze serum glucose and serum insulin (4 mL of blood), and another with ethylenediamine tetraacetic acid (EDTA) anticoagulant to analyze glycated hemoglobin.

Plasma was separated from the whole blood by centrifugation at 1831xg during 15 minutes at 4°C (Centrifuge CIENTEC® 4K15, United Kingdom). After that, we extracted plasma using an automatic pipette, added to demineralize polypropylene micro tubes, and stored at -20°C for later analysis (freezer Brastemp, model BVR28/220, Sao Paulo, Brazil). We used methods proposed by Whitehouse et al.²⁸ to separate erythrocyte. Red cell mass was transferred to demineralize polypropylene micro tubes which were maintained at -20°C for later analysis.

We performed elemental analyses of the magnesium and calcium using an inductively coupled plasma spectrometer - Optical Emission Spectrometry with an axial view configuration and a V-Groove nebulizer (720 ICP / OES, Varian Inc., California, USA). Reference values adopted were 0.75 to 1.05 mmol/l for plasma magnesium²⁹, 1.65 to 2.65 mmol/l for erythrocyte magnesium, 3.00 to 5.00 for mmol/day for urinary magnesium³⁰, 2.12 to 2.65 mmol/l for plasma calcium³¹, and lower values than 4.0 mg/kg/day for urinary calcium³².

Assessment of Ca/Mg Ratio

We calculated the Ca/Mg ratios in diet, plasma and urine using the formulas: Ca/Mg dietary ratio=dietary Ca intake (mg/day)/ dietary Mg intake (mg/day); Ca/Mg plasma ratio=plasma Ca (mmol/l)/ plasma Mg (mmol/l) and Ca/Mg urinary ratio=urinary Ca (mmol/day)/ urinary Mg (mmol/day).

Determination of Glycemic Parameters

Fasting serum glucose was measured using the colorimetric enzymatic method and Labtest kits. We considered values between 75 and 99 mg/dl as normal³³. Serum insulin concentration was determined using the chemiluminescence method. Normal ranges of insulin were considered for values between 6-27 µU/ml. Analysis of glycated hemoglobin was carried out using the ion exchange chromatography method. We considered values lower than 5.7% as normal³³.

Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from fasting serum glucose and fasting serum insulin. HOMA-IR values greater than 2.71 were classified as insulin resistance^{34,35}. We have calculated HOMA-β index using the HOMA Calculator version 2.2.3 program. (HOMA Calculator, 2017). We considered HOMA-β values greater than 1.8 as not normal^{34,35}.

Statistical Analysis

Treatment of missing data

During data consolidation, we observed that the mean percentage of missing data was low (8.4%). However, the variables with missing values had a mean of 21.0% in control group. Thus, we implemented an imputation process for missing-data to “complete” the database. We used a simple imputation method to treat variables groups with 15% missing data or fewer, and we used a multiple imputation method to treat variables groups with missing data higher than 15%, according to Harrel³⁶.

We carried out a simple and multiple imputation methods by Predictive Mean Matching (PMM) method. Simple imputation was conducted in three steps³⁷: (1) estimation of regression model-the response variable was the variable of interest (to be imputed), and the others variables were explanatory; (2) estimation of variable of interest value of subjects with missing data, and (3) pairing the predicted value of the variable of interest with nearest adjusted value of subjects with missing data (we calculated the Euclidean distances). We imputed the value corresponding to the nearest adjusted value. If there was more than one adjusted value with distance equal to the minimum distance found, the imputed value was chosen randomly among the equal values.

Multiple imputation from PMM was based on the same principles of the simple imputation method, but many values were generated, not only one. After multiple imputations have been made with $m=5$, resulting in 5 “complete” data sets, the general estimates more accurate of values were obtained by the mean of values imputed.

We used a function created by Andreozzi³⁸ for simple imputations, and we used the multivariate imputation by chained equations (MICE) software for multiple imputations. We performed the imputation process in R³⁸.

Data analysis

The Kolmogorov-Smirnov test was used to check the normality assumption of the variables. We compared the means of continuous variables using the Student’s t-test for normally distributed variables, whereas the Mann-Whitney test was performed for non-normally distributed variables. The Pearson chi-square test was used to compare categorical variables and we have performed Spearman’s Rho (ρ) for analysis of correlations between variables. A p value <0.05 was significant.

Canonical correlation analysis was performed to check associations between mineral group (dietary magnesium intake, plasma magnesium, erythrocyte magnesium, urinary magnesium, dietary calcium intake, plasma calcium and urinary calcium) and glycemic control parameters group (fasting glucose, serum insulin and glycated hemoglobin). Data were normalized to have means equal to zero and standard deviations equal to one in order to reduce the influence of a set of high values variables on another set of low values variables. We did not included the HOMA-

IR and HOMA- β in canonical correlation analysis due to multicollinearity detected.

We performed the Wilks’ lambda (F approximation to the distribution) to evaluate significance of canonical roots. Explained variance was calculated by squared canonical correlation (canonical R²). Canonical charges and cross-canonical charges were estimated.

We performed logistic regression models to evaluate associations between HOMA-IR and magnesium parameters (dietary magnesium intake, plasma magnesium, erythrocyte magnesium and urinary magnesium) and Ca/Mg ratio, separately, with each variable categorized into quartiles with the lowest quartile as the referent category. A final model with dietary magnesium intake was adjusted for age, dietary calcium intake, plasma calcium and urinary calcium, whereas the final model with Ca/Mg ratio was adjusted for age.

RESULTS

Table 1 shows the mean values and standard deviations of age, anthropometric parameters, and glycemic parameters in women with obesity and normal weight. Statistically significant difference ($p<0.05$) between obese and normal weight groups in body weight, height, BMI and WC was found. There were no differences in fasting serum glucose, serum insulin, glycated hemoglobin, HOMA-IR and HOMA- β between groups.

Women with obesity had intakes of magnesium and calcium below the EAR. There were differences in daily magnesium intake between the obese and normal-weight groups ($p<0.05$), but the daily calcium intake was not significantly different between two groups. However, dietary magnesium and calcium intake per kilogram of body weight per day was lower in obese women than control group ($p<0.001$). Women with obesity had lower plasma and erythrocyte magnesium, lower plasma calcium, and higher urinary concentrations of magnesium and calcium than control group did ($p<0.001$). There were significant differences in Ca/Mg plasma ratio and Ca/Mg urinary ratio between the women with obesity and normal-weight women ($p<0.05$), but Ca/Mg intake ratio was not different (Table 2).

A statistically significant association between obesity and decreased erythrocyte magnesium, and between obesity and reduced plasma calcium was found ($p<0.001$). Furthermore, a statistically significant association between obesity and increased urinary excretion of magnesium and calcium was found ($p<0.001$; Pearson chi-squared test (data not shown).

A statistically significant correlation was found between dietary magnesium and plasma calcium, erythrocyte magnesium and dietary calcium, urinary magnesium and urinary calcium in obese participants. Statistically significant correlation was also found between dietary magnesium and dietary calcium, erythrocyte magnesium and urinary calcium in the control group (Table 3).

A statistically significant correlation was found between

Ca/Mg intake ratio and HbA1c, urinary calcium and serum insulin, urinary calcium and HOMA-IR, urinary calcium and HOMA- β , urinary calcium and HbA1c in obese participants (Table 4). There was a statistically significant correlation between urinary calcium and fasting glucose in the control group ($p < 0.05$) (data not shown in table).

The first canonical function was significant, and the correlation between variable sets of minerals and variable sets of glycemic parameters was expressive. Urinary calcium received a greater canonical charge (0.934) than did other parameters of minerals in construction of the first mineral canonical variable. Glycated hemoglobin (-0.638) had the greatest impact in the variable sets of glycemic parameters (Table 5).

Logistic regression analysis did not show a significant association between magnesium status and HOMA-IR, nor between Ca/Mg ratio and HOMA-IR (data not shown in table). However, the odds of increased Ca/Mg urinary ratio was 1.69 higher in women with insulin resistance and obesity compared to non-insulin resistant obese women (Table 6).

DISCUSSION

In this study, we evaluated magnesium and calcium status and the association between Ca/Mg ratio and insulin resistance. Plasma and erythrocyte magnesium concentrations were lower in obese women compared to normal-weight women, and lower than reference values.

Additionally, a significant association between obesity and reduced erythrocyte magnesium concentrations was found. Guerrero et al.⁶ also found reduced serum magnesium in obese patients.

There are several contributing factors to magnesium deficiency in obese patients. In this study, reduced dietary intake of magnesium may have contributed to reduced blood magnesium concentrations because the dietary intake of magnesium was lower than dietary reference intakes. Furthermore, dietary magnesium intake per kilogram of body weight was also lower in obese women compared to the control group. The Brazilian Household Budget Survey (2008-2009)³⁹ showed that Brazilian population in general does not have the habit of eating magnesium-containing foods, for example leafy green vegetables and nuts.

Elevated urinary magnesium excretion verified in obese women may also explain magnesium deficiency. We found a significant association between obesity and elevated urinary magnesium excretion, thus evidencing altered magnesium homeostasis in obese patients. Therefore, the reduced dietary intake of magnesium and elevated urinary magnesium excretion may together explain reduced blood magnesium concentration in obese women.

Calcium can also regulate magnesium homeostasis. In this study, there was significant negative correlation between dietary calcium intake and erythrocyte magnesium concentration in obese women. Accordingly, we suggest that if the dietary intake of calcium increases, the erythrocyte

Table 1. Mean values and standard deviations of age, anthropometric parameters, and glycemic control parameters of participants.

Parameters	Obese women (n=62) Mean $\pm$ SD	Control group (n=66) Mean $\pm$ SD	p-value
Age (years)	34.0 $\pm$ 8.8	36.0 $\pm$ 7.9	0.16
Body weight (kg)	104 $\pm$ 10.3	56.0 $\pm$ 5.7	< 0.001*
Height (m)	1.61 $\pm$ 0.06	1.58 $\pm$ 0.07	0.04*
BMI (kg/m ²)	40.3 $\pm$ 3.4	22.4 $\pm$ 1.7	< 0.001*
WC	111 $\pm$ 9.4	74.7 $\pm$ 5.3	< 0.001*
Fasting glucose (mg/dL)	83.6 $\pm$ 12.9	79.7 $\pm$ 8.5	0.15
Serum insulin (μ U/mL)	11.1 $\pm$ 3.9	9.51 $\pm$ 2.0	0.08
HbA1c (%)	5.17 $\pm$ 0.49	5.01 $\pm$ 0.56	0.20
HOMA-IR	2.37 $\pm$ 1.13	1.88 $\pm$ 0.46	0.06
HOMA- β	1.42 $\pm$ 0.53	1.19 $\pm$ 0.25	0.07

*Values significantly different between obese women and control groups, Student t test or Mann-Whitney test ($p < 0.05$). BMI = body mass index; WC = waist circumference; HbA1c = glycated hemoglobin; HOMA-IR = Homeostasis Model Assessment Insulin Resistance; HOMA- β = Homeostasis Model Assessment β ; SD = standard deviations. Reference values: Fasting Glucose = 75 to 99 mg / dL; Serum insulin = 6 to 27 μ U / mL; HbA1 <5.7; HOMA-IR < 2.71; HOMA- β < 1.8.

Table 2. Mean values and standard deviations of parameters of magnesium and calcium of obese and normal-weight groups.

Parameters	Obese women (n= 62) Mean±SD	Normal-weight women (n= 66)	p-value
		Mean±SD	
Dietary Mg (mg/day)	186±26.4	178±30.5	0.01*
Dietary Mg (mg/kg/day)	1.80±0.25	3.21±0.62	<0.001*
Plasma Mg (mmol/l)	0.61±0.11	0.92±0.10	<0.001*
Erythrocyte Mg (mmol/l)	1.57±0.12	2.31±0.24	<0.001*
Urinary Mg (mmol/day)	6.02±1.96	3.45±1.60	<0.001*
Dietary Ca (mg/day)	542±52.5	548±108	0.69
Dietary Ca (mg/kg/day)	5.26±0.63	9.88±2.15	<0.001*
Plasma Ca (mmol/l)	1.94±0.18	2.46±0.20	<0.001*
Urinary Ca (mg/kg/day)	5.45±2.56	2.96±0.92	<0.001*
Ca/Mg intake ratio	2.98±0.56	3.14±0.70	0.10
Ca/Mg plasma ratio	3.28±0.61	2.71±0.39	<0.001*
Ca/Mg urinary ratio	2.50±1.32	1.44±0.75	<0.001*

* Significantly different values between obese and normal-weight women, Student t test (p <0.05). Mg = magnesium; Ca = calcium; SD = standard deviations.

Table 3. Simple linear correlation analysis between magnesium and calcium parameters in obese and normal-weight women.

Parameters	Obese women						Control group					
	Dietary Ca		Plasma Ca		Urinary Ca		Dietary Ca		Plasma Ca		Urinary Ca	
	r	p	r	p	r	p	r	p	r	p	r	p
Dietary Mg	0.24	0.06	-0.33	0.01*	-0.21	0.10	0.29	0.02*	0.01	0.96	-0.12	0.34
Plasma Mg	0.10	0.45	-0.13	0.31	-0.18	0.15	-0.05	0.67	-0.04	0.78	0.07	0.59
Erythrocyte Mg	-0.47	<0.001*	0.12	0.38	0.20	0.11	0.22	0.08	-0.04	0.77	-0.44	<0.001*
Urinary Mg	0.07	0.60	0.22	0.08	0.43	<0.001*	0.06	0.66	-0.08	0.54	0.17	0.18

Pearson correlation coefficient or Spearman correlation coefficient (p <0.05). Mg= magnesium; Ca= calcium.

Table 4. Simple linear correlation analysis between magnesium and calcium parameters and glycemic control parameters in obese women.

Parameters	Fasting glucose		Serum insulin		HbA1c		HOMA-IR		HOMA-β	
	r	p	r	p	r	p	r	p	r	p
Dietary Mg	0.10	0.42	0.09	0.49	0.21	0.10	0.09	0.48	0.09	0.47
Plasma Mg	0.05	0.70	0.01	0.96	0.11	0.39	0.03	0.83	0.02	0.87
Erythrocyte Mg	0.04	0.74	0.05	0.69	0.10	0.45	0.04	0.73	0.04	0.77
Urinary Mg	-0.10	0.42	0.11	0.41	0.05	0.69	0.06	0.66	0.10	0.46
Dietary Ca	-0.02	0.85	-0.20	0.12	-0.02	0.87	-0.17	0.20	-0.19	0.14
Plasma Ca	-0.17	0.19	0.01	0.95	-0.23	0.08	0.01	0.96	-	0.10
Urinary Ca	-0.06	0.64	0.34	0.01*	-0.29	0.02*	0.26	0.04*	0.32	0.01*
Ca/Mg intake ratio	-0.20	0.12	-0.19	0.13	-0.30	0.02*	-0.20	0.13	-0.20	0.12
Ca/Mg plasma ratio	-0.09	0.47	0.03	0.83	-0.18	0.15	0.01	0.93	0.01	0.92
Ca/Mg urinary ratio	0.05	0.69	0.14	0.27	-0.19	0.14	0.13	0.32	0.14	0.27

Pearson correlation coefficient or Spearman correlation coefficient ($p < 0.05$). Mg= magnesium; Ca= calcium; HbA1c= glycated hemoglobin; HOMA-IR= Homeostasis Model Assessment Insulin Resistance; HOMA-β= Homeostasis Model Assessment β.

Table 5. Canonical correlation between minerals and parameters of control glycemic in obese women.

Canonical function	Canonical correlation	Canonical R ²	Fa	GL ₁	GL ₂	p
1	0.64	0.42	2.00	21.0	150	0.01
2	0.35	0.12	0.78	12.0	106	0.67
3	0.20	0.04	0.47	5.00	54	0.80
Canonical charges and cross canonical charges for the canonical function 1						
Parameters	Canonical charges		Cross canonical charges			
Dietary Mg (mg/day)	-0.26		-0.16			
Plasma Mg (mmol/l)	-0.17		-0.11			
Erythrocyte Mg (mmol/l)	-0.06		-0.04			
Urinary Mg (mmol/day)	-0.05		-0.03			
Dietary Ca (mg/day)	-0.26		-0.16			
Plasma Ca (mmol/l)	0.31		0.20			
Urinary Ca (mg/day)	0.93		0.60			
Parameters of glycemic control						
Fasting glucose (mg/dL)	-0.12		-0.08			
Serum insulin (μU/mL)	0.45		0.29			
HbA1c (%)	-0.64		-0.41			

Wilks' Lambda multivariate test. Fa: Approximate F; GL1: degrees of freedom from the samples; GL2: degrees of freedom regarding error. Mg = magnesium; Ca = calcium; HbA1c = glycated hemoglobin.

Table 6. Odds ratio (OR) for the relationship between Ca/Mg ratio and insulin resistance in the non-insulin resistant and insulin resistant obese women.

Model	Parameters	OR	95%IC	p
1	Ca/Mg intake ratio	0.62	0.16 – 2.40	0.49
2	Ca/Mg plasma ratio	0.81	0.31 – 2.12	0.67
3	Ca/Mg urinary ratio	1.69	1.06 – 2.68	0.03*

All models adjusted for BMI and WC.

magnesium concentration decreases in obese women, favoring magnesium deficiency. Bertinato et al.⁹ suggests that small increases in dietary calcium exacerbate magnesium deficiency in rats fed an inadequate magnesium diet by reducing intestinal magnesium absorption and by impairing renal magnesium reabsorption.

This result may be justified because the homeostatic regulating system of calcium and magnesium involve the same transporters in intestinal and renal cells, for example the Calcium Sensing Receptor (CaSR), Transient Receptor Potential Melastatin (TRPM) 6 and TRPM7. Thus, calcium and magnesium antagonize each other in many physiological activities^{7,8}. It is noteworthy that calcium absorption occurs via an active transcellular transport when intestinal calcium concentration is low, and the magnesium absorption occurs in the same way. Therefore, in a low-intake condition, magnesium and calcium may compete even more for these transporters⁷.

Moreover, evidence reviewed by Seelig⁴⁰ indicates that the greatest average daily magnesium loss occurs in men on very low magnesium intakes (<4 mg/kg/d) and low calcium intakes. In our study, obese women had intakes of magnesium below this value (1.80±0.25 mg/kg/d). This fact may be associated with low intestinal magnesium absorption in obese women, inducing magnesium deficiency.

Another important result was that Ca/Mg intake ratio was elevated in women with obesity compared to recommended values by population-based cohort studies conducted in China (value between 1.7 and 2.8)¹⁰, and in relation to recommended values by Durlach⁴¹ for optimal health in adult population (Ca/Mg intake ratio close to 2). Therefore, the increased Ca/Mg intake ratio suggests there was, probably, high Ca/Mg intestinal ratio and, this way, more calcium to compete with magnesium in enterocytes.

It has also been postulated that calcium may indirectly affect magnesium absorption through changes in membrane permeability because intestinal membrane permeability is sensitive to alterations in luminal calcium and magnesium concentrations. However, further investigation is required to verify its importance in short and long-term regulation of calcium and magnesium transport^{42,43,44}.

On the other hand, magnesium can also affect calcium

absorption by competing for intestinal transporters and through changes in membrane permeability^{8,43}. A significant negative correlation between dietary magnesium intake and plasma calcium concentrations in obese women found in this study may reflect this fact. Thus, this result suggests magnesium also influences calcium metabolism.

Studies have shown the biochemical relevance of the Ca/Mg intake ratio and the Ca/Mg serum ratio in healthy and sick individuals^{10,14,45}. In our study, the Ca/Mg plasma ratio and the Ca/Mg urinary ratio were higher in obese women compared to normal-weight women. Regarding increased Ca/Mg plasma ratio, inflammation, oxidative stress and insulin resistance are the main metabolic disorders associated with imbalance between magnesium and calcium in blood^{10,13,46}. However, there was no significant correlation between Ca/Mg plasma ratio and glycemic parameters in this study. This result may be explained because obese women had adequate glycemic control and others variables may have regulated the glycemic parameters in obese women.

In this study, we found a significant negative correlation between Ca/Mg intake ratio and glycated hemoglobin among obese women. This result is opposite our hypothesis and incompatible with the evidence found in the published literature. Because the increase of Ca/Mg intake ratio is associated with glycemic decontrol and disease mortality^{10,45}. Haenni et al.¹⁴ have shown that changes in insulin sensitivity index were inversely correlated to changes in Ca/Mg serum ratio. Thus, further studies should be carried out.

Using odds ratio analysis to better understand the association between the Ca/Mg ratio and insulin resistance, we observed that the odds of increased Ca/Mg urinary ratio was 1.69 higher in insulin resistant obese women compared to non-insulin resistant obese women. Our study is the first to determinate the Ca/Mg urinary ratio in insulin resistant obese patients. However, other research studies had already evaluated Ca/Mg intracellular ratio, and experimental results suggest that a higher intracellular ratio of Ca/Mg may lead to hypertension and insulin resistance^{47,48,49}.

Some limitations of this study should be mentioned, for example, the cross-sectional design, the small sample size, and the fact that intracellular calcium concentration was not determined. Another limitation is we did not determine

expression of the gene encoding the CaSR and TRPM7 receptors. A strength of our study is this research was the first to associate the Ca/Mg ratio in diet, plasma and urine with insulin resistance in women with obesity.

CONCLUSION

Women with obesity in this study shows reduced dietary intake of magnesium, reduced plasma and erythrocyte magnesium concentrations, and elevated urinary magnesium excretion. Plasma calcium concentration is lower in patients with obesity and urinary calcium excretion are higher than in the normal-weight group. Dietary magnesium and calcium intake per kilogram of body weight per day was lower in obese women than in the control group. Plasma and urine ratio of Ca/Mg in obese women was increased.

Simple linear correlation analysis showed that calcium seems to influence magnesium homeostasis, as increased dietary calcium intake may be associated to reduced erythrocyte magnesium concentration. Ca/Mg ratio seems to be associated with insulin resistance in obese women. Therefore, further research should be conducted to clarify the relevance of the Ca/Mg ratio in obesity.

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