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Influence of magnesium on biochemical parameters of iron and oxidative stress in patients with type 2 diabetes

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Abstract

Introduction: **Studies have shown that oxidative stress, found in patients with type 2 diabetes, may be due to changes in the metabolism of minerals, such as magnesium and iron. Data related to compartmentalization of these minerals in diabetes are scarce and controversial.**

Objective: **This study assessed the influence of magnesium on biochemical parameters of iron and oxidative stress in patients with type 2 diabetes.**

Methods: **A case-control study in male and female subjects aged 27-59 years, divided into two groups: type 2 diabetes (n=40) and control (n=48). Intake of magnesium and iron was assessed by three-day food record. Plasma, erythrocyte and urinary levels of magnesium, serum iron, ferritin, total iron binding capacity, fasting glucose, glycated hemoglobin, insulin, creatinine clearance and plasma thiobarbituric acid reactive substances (TBARS) were analyzed.**

Results and Discussion: **Magnesium intake and plasma magnesium were lower in diabetic subjects. There was low urinary magnesium excretion, with no difference between groups. Although normal, the diabetic group had lower serum iron and ferritin concentrations compared to control subjects. Plasma TBARS in diabetic patients was higher than control while creatinine clearance was lower. An inverse correlation between erythrocyte magnesium and serum iron and ferritin was observed in the diabetes group.**

Conclusions: **Diabetes induced hypomagnesemia and this, associated with chronic hyperglycemia, may have enhanced oxidative stress. Erythrocyte magnesium may have contributed to prevent iron overload and worsening of oxidative stress and hyperglycemic status.**

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Key words: *Diabetes. Iron. Magnesium. Oxidative stress.*

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INFLUENCIA DE MAGNESIO EN LA BIOQUÍMICA DEL HIERRO Y EL ESTRÉS OXIDATIVO EN PACIENTES CON DIABETES TIPO 2

Resumen

Introducción: **Los estudios han demostrado que el estrés oxidativo, que se encuentra en pacientes con diabetes tipo 2, puede ser debido a cambios en el metabolismo de los minerales, como el magnesio y el hierro. Los datos relacionados con la compartimentación de estos minerales en la diabetes son pocos y cuestionables.**

Objetivos: **Evaluar la influencia del magnesio sobre parámetros bioquímicos de hierro y el estrés oxidativo en pacientes con diabetes tipo 2.**

Métodos: **Estudio caso-control en los sujetos masculinos y femeninos de edad 27 a 59 años, divididos en dos grupos: la diabetes tipo 2 (n = 40) y control (n = 48). La ingesta de magnesio y hierro se evaluó por tres días registro de alimentos. Plasma, eritrocitos y los niveles urinarios de magnesio, hierro sérico, ferritina, capacidad total de fijación del hierro, glucosa en ayunas, hemoglobina glucosilada, la insulina, el aclaramiento de creatinina y el plasma se analizaron tiobarbitúrico sustancias reactivas al ácido (TBARS).**

Resultados y Discusión: **La ingesta de magnesio y el magnesio en plasma fueron más bajos en los pacientes diabéticos. Hubo baja excreción urinaria de magnesio, sin diferencias entre los grupos. Aunque lo normal, el grupo de diabéticos tenían concentraciones de hierro y ferritina sérica inferiores en comparación con los sujetos control. TBARS plasmáticos en los pacientes diabéticos fue mayor que en el control, mientras que la depuración de creatinina fue menor. Se observó una correlación inversa entre el magnesio y el hierro en suero de los eritrocitos y la ferritina en el grupo de diabetes**

Conclusiones: **Diabetes hipomagnesemia inducida y esto, asociado a la hiperglucemia crónica, pueden haber mejorado el estrés oxidativo. Magnesio eritrocitaria puede haber contribuido a evitar la sobrecarga de hierro y el empeoramiento de estrés oxidativo y el estado de hiperglucemia.**

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Palabras clave: *Diabetes. Hierro. Magnesio. Estrés oxidativo.*

Abbreviations

BMI: Body Mass Index. Page 8.
TBARS: Thiobarbituric acid reactive substances. Page 4, 5, 10, 11, 14, 15.
HbA1: glycated hemoglobin. Page 9.
TIBIC: Total iron binding capacity. Page 9, 12.
HOMA: Homeostasis model assessment. Page 10.

Introduction

Type 2 diabetes mellitus is a chronic non-communicable disease characterized by the presence of metabolic disorder, high hyperglicemia, dyslipidemia and endothelial dysfunction, and the main pathophysiological change is insulin resistance[1,2]. Chronic hyperglycemia found in this disease contributes to the development of oxidative stress associated with increased production of reactive oxygen species and lipid peroxidation[3].

Oxidative stress may occur due to changes in plasma and intracellular concentrations of some minerals, such as magnesium and iron[4,5]. Magnesium is the second most abundant cation in the intracellular environment, and is used as a cofactor in over 300 enzymatic reactions, including those producing or using magnesium-ATP complex. Deficiency of this mineral in patients with type 2 diabetes has been attributed to elevated urinary excretion induced by hyperglycemia, decreased intestinal absorption and reduced concentration of this mineral in diet[6].

Some mechanisms have been proposed to clarify the relationship between magnesium deficiency and iron metabolism in patients with type 2 diabetes. Accordingly, results of some studies suggest that such deficiency is involved in the induction of hemolysis, contributing to iron release which is, in turn, a pro-oxidant nutrient enhancing the production of reactive oxygen species and the synthesis of inflammatory markers[5,7].

Iron is a vital mineral for cell homeostasis, which activity is linked to chemical characteristics and oxidation state, with participation in Fenton and Haber-Weiss reactions. High iron levels induce the production of free radicals which promote DNA damage and interact with unsaturated fatty acids, inducing lipid peroxidation[8].

To test the hypothesis that the magnesium status is altered in patients with type 2 diabetes, and this alteration may influence on biochemical parameters of iron and oxidative stress, especially in patients with type 2 diabetes, this study was done.

Material and Methods

This was a case-control study involving 88 male and female subjects aged between 27-59 years, divided into the following groups: type 2 diabetes (n = 40) and control (n = 48). Diabetic and healthy control subjects were consecutively screened through interviews, between March and August 2012. The eligibility criteria was as follows: clinical treatment with oral hypoglycemic drugs only, no diabetic complications (renal failure, neuropathy, cataract), non-pregnant and non-breastfeeding women, non-smokers, no recent history of use of the following: alcohol, vitamin-mineral supplement and/or other medications that could interfere with magnesium and iron nutritional status assessment. The patients assessed in the present study were attended by a health team of primary care units, and they had been diagnosed with type 2 diabetes by doctors, according to the criteria set by American Diabetes Association[1,9]. The same criteria were applied in the control group, except medical diagnosis of type 2 diabetes. The control group consisted of volunteers attended by a health team of primary care units with similar characteristics to the case group in terms of age and socioeconomic status. Furthermore, they had not family history of diabetes or other chronic diseases. The selection of the control group was performed considering the proportion of one control for each case. The study was approved by the Research Ethics Committee of Federal University of Piauí (CAAE protocol #0156.0.045.000-11).

The sample size of the study was based on the selection criteria and in the number of individuals with type 2 diabetes mellitus registered in basic health units from Teresina-PI. It was based on the universe of 150 patients and was adopted confidence interval of 95%, a margin of error of 5%, considering the 7.0% prevalence of type 2 diabetes mellitus in the Northeast, totaling a minimum sample of 61 patients. However, at the end of the study, the total sample was 40 type 2 diabetic patients, due to losses that occurred by age (elderly), presence of associated diseases, such as hepatic steatosis, and use of insulin and/or vitamins and minerals supplements.

Assessment of nutritional status

Body mass index (BMI) of the participants was calculated dividing weight in kilograms by height squared in meters. Nutritional status was classified based on BMI, according to World Health Organization recommendations[10].

Determination of usual magnesium and iron in diet

Dietary intake was assessed using a three-day food record, including two days during the week and one day on the weekend (Saturday or Sunday). Nutritional assessment was performed using Nutwin software, version 1.5[11]. Methods and recommendations outlined in the Dietary Reference Intakes were employed to estimate magnesium and iron intake[12,13].

Determination of biochemical parameters for magnesium

Blood samples (25 mL) were collected in the morning, after fasting of 12-14 hours. Blood was distributed into: a demineralized glass tube containing 30% sodium citrate as anticoagulant (10 μL/mL blood) to analyze magnesium levels; a vacutainer tube containing EDTA to analyze thiobarbituric acid reactive substances (TBARS) and glycated hemoglobin (HbA1); and the remainder was placed in a tube with no anticoagulant for other analyses.

Urine sample was collected 24 hours prior to blood collection in a demineralized flask supplied by the researchers.

Concentrations of magnesium in plasma, urine and erythrocytes were determined by flame atomic absorption spectrometry (AAnalyst 100; Perkin Elmer, Norwalk, CT, USA) as methodology previously standardized and validated[14]. All materials used for collection and mineral analysis were previously demineralized and the method precision and accuracy were checked using certified standards (Trace Elements Serum L-I and Urine L-I; Seronorm, Billingstad, Norway), and secondary standards for plasma, urine and erythrocytes. The reference values were the following: (a) plasma: 0.75-1.05 mmol/L [15] (b) urine: 3.00-5.00 mmol/d[16]; (c) erythrocytes: 1.65-2.65 mmol/L[16].

Determination of biochemical parameters for iron

Biochemical parameters for serum iron were determined using kits from Labtest Diagnóstica (Lagoa Santa, MG, Brazil). The reference values were those indicated in the kits. Ferritin was determined using chemiluminescence method (reference values: 28-397 ng/mL for male adults and 6-159 ng/mL for female adults), serum iron by colorimetric method (reference value: 59-158 µg/dL for male and 37-145 µg/dL for female) and total iron binding capacity (TIBIC) by modified Goodwin method (reference value suggested by the manufacturer: 250-410 µg/dL).

Determination of glycemic control and renal function assessment

Fasting glucose was determined by colorimetric-enzymatic method, using kit from Abbott Laboratories (Abbott Park, IL, USA). For fasting glucose, normal values ranged from 75-99 mg/dL and altered values were considered ≥ 126 mg/dL diagnosed with diabetes mellitus[1,9].

Glycated hemoglobin was determined using turbidimetric immunoassay method (reference values: 4-6 %[9]),and insulin levels were determined by chemiluminescence method (reference values between 6-27 µU/mL). For both analyses, kits from Abbott Laboratories were used.

Insulin resistance was calculated by means of the homeostasis model assessment (HOMA) index, defined by the equation HOMA = fasting glucose (mmol/L) x fasting insulin (µU/mL)/22.5.

Creatinine clearance was calculated by Cockcroft-Gault formula[17] and the results corrected by body surface area.

Determination of thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances (TBARS) in plasma were determined using the method suggested by Ohkawa; Ohishi; Yagi[18]. Prior to sample processing, analytical calibration curve was prepared at concentrations of 0.5; 1.0; 2.0; 4.0 and 8.0 nmol/mL, using 1,1,3,3-tetraethoxypropane as standard. Absorbance was read using a UV/Vis Bel Photonics spectrophotometer, SP 1102 model (Osasco, SP, Brazil) at a wavelength of 532 nm.

Statistical analysis

Data was analyzed using statistical software SPSS for Windows 15.0. To differentiate parametric from non-parametric values, Kolmogorov-Smirnov normality test was employed. To compare the groups, Student t-test was used for parametric values and Mann-Whitney test was used for non-parametric values, with a significance level of $p<0.05$. Pearson's correlation coefficient was used to check the potential interrelationship between variables.

In order to reduce errors associated with dietary assessment, intake values were adjusted for total energy intake using the residual method, and the intraindividual variation, calculated by the mean of the components for variance analysis.

Results

The mean age was 51.8 ± 5.1 (39-59) years and 40.5 ± 8.9 (27-58) years in type 2 diabetes (n = 40) and control (n = 48) groups, respectively. The duration of diabetes was 7.2 ± 3.4 years. The mean body mass index was 28.6 ± 4.6 kg/m^2 in type 2 diabetes and 23.6 ± 3.4 kg/m^2 in control group.

Overall, the glycemic control was unsatisfactory in type 2 diabetic subjects, with higher insulin resistance compared to control group and diabetic patients had higher plasma TBARS levels (table I).

The study shows the mean and standard deviation values for creatinine clearance was lower in the diabetic subjects (71.52 ± 12.05 mL/min/1.73m^2) compared to controls (99.05 ± 28.79 mL/min/1.73m^2). On the

Table I
Glycemic control and thiobarbituric acid reactive substances (TBARS) of subjects with type 2 diabetes and control groups

Parameters	*Type 2 diabetes (n = 40)*	*Control (n = 48)*	*p*
Fasting serum glucose (mg/dL)	178.53 ± 32.63	82.77 ± 9.20	<0.001
Glycated hemoglobin (%)	7.68 ± 1.60	5.13 ± 0.63	<0.001
Fasting serum insulin (μU/mL)	32.67 ± 16.13	25.08 ± 13.4	0.018
HOMA-IR (%)	15.19 ± 10.07	5.12 ± 2.75	<0.001
Plasma TBARS (nmol/L)	2.40 ± 0.97	1.81 ± 0.67	<0.001

The results are expressed in the form of mean ± standard deviation. Data compared by Student t-test. HOMA-IR: homeostasis model assessment of insulin resistance. Reference values: fasting glucose = 75-99 mg/dL[9]; glycated hemoglobin = 4-6 %[9]; fasting serum insulin = 6-27 μU/mL.

other hand, urinary output was higher for type 2 diabetics (1933 ± 779.5 mL/d) compared to controls (1186 ± 548.7 mL/d). A statistically significant difference was seen in the creatinine clearance and urinary volume parameters between groups (p<0.05).

Table II shows the mean and standard deviation values for magnesium concentration in the diet. There was a mean intake of 10.40 ± 1.24 mg/d and 8.41 ± 1.46 mg/d for diabetics and healthy women, respectively. In addition, intake was observed for 10.19 ± 1.26 mg/d diabetic men and 8.22 ± 1.05 mg/d healthy. The mean intake of magnesium in both groups was low and a statistically significant difference between groups (p<0.05).

The mean concentration of magnesium in plasma was lower in diabetic subjects, and below the lower reference limit. Despite the absence of difference between groups, urinary excretion of magnesium was low in both groups. No difference was found in erythrocyte magnesium levels, nor values outside recommendation (table II).

Sixty-three percent of the diabetes' group and 31 % of the controls had plasma magnesium below the 0.75 mmol/L. Eighty-three percent and 75 % of diabetes' group and controls, respectively, excreted less than 3 mmol/L. On the other hand, none had erythrocyte magnesium below 1.65 mmol/L.

Lower serum levels of iron and ferritin were found in the diabetic group compared to control subjects, and the difference in serum iron was more evident among male subjects, while this was observed in ferritin levels among female subjects. A difference was observed in TIBIC only in the male subjects (table III).

Table IV shows that an inverse relationship between erythrocyte magnesium and serum levels of iron and ferritin was found in the diabetes group, and direct relationship between plasma magnesium and iron in the control group.

Discussion

In this study a significant frequency of hypomagnesemia was found in the diabetic patients, and the mean concentration of this mineral in plasma was lower than control group. This is a common observation in studies assessing patients with type 2 diabetes[19,20,21].

Overall, there was a high probability of inadequate dietary intake of magnesium, which can be explained by low consumption of foods rich in magnesium, such as whole grains, nuts, almonds, and dark green vegetables[12], especially in the control group.

Although diabetic subjects had a higher intake of magnesium compared to the control group, the lower

Table II
Magnesium status of subjects with type 2 diabetes and control groups

Parameters	*Type 2 diabetes (n = 40)*	*Control (n = 48)*	*p*
Magnesium intake (mmol/d)[a]	10.28 ± 1.24	8.36 ± 1.34	< 0.001
Plasma magnesium (mmol/L)	0.72 ± 0.09	0.78 ± 0.08	0.005
Erythrocyte magnesium (mmol/L)	2.29 ± 0.29	2.25 ± 0.48	0.638
Urine magnesium excretion (mmol/d)	2.08 ± 1.18	2.00 ± 1.08	0.756

The results are expressed in the form of mean ± standard deviation. Data compared by Student t-test. Reference values: plasma magnesium = 0.75-1.05 mmol/L[15]; erythrocyte magnesium = 1.65-2.65 mmol/L [16]; urine magnesium excretion = 3.00-5.00 mmol/d[16]. [a]Data adjusted according to the individual energy intake and intra-individual variability. The estimated average requirements are: a) for males: 14.4 mmol/d (>30 years old); b) for females 10.9 mmol/d (>30 years old).

Table III
Iron status of subjects with type 2 diabetes and control groups

Parameters	Type 2 diabetes		Control	
	Male Mean ± SD	Female Mean ± SD	Male Mean ± SD	Female Mean ± SD
Iron intake (mg/d)[a]	12.4 ± 1.36	11.6 ± 1.07	12.4 ± 2.06	12.0 ± 3.46
Serum iron (µg/dL)	62.56* ± 22.10	65.59 ± 23,93	120.13* ± 43.27	78.57 ± 25.39
Ferritin (µg/dL)	34.46 ± 18.36	38.64* ± 19.92	39.36 ± 20.50	67.84* ± 27.78
TIBIC (µg/dL)	307.56* ± 46.11	304.04 ± 55.79	272.13* ± 13.0	321.29 ± 48.61

Data compared by Student t-test. TIBIC: total iron-binding capacity. Reference values: serum iron = 59-158 µg/dL (for male) e 37-145 µg/dL (for female); ferritin = 6-159 µg/dL (for male) e 28-397 µg/dL (for female); TIBIC = 250-410 µg/dL. [a]Data adjusted according to the individual energy intake and intra-individual variability. The estimated average requirements are: a) for males: 6.0 mg/d b) for females: 8.1 mg/d

plasma levels of this mineral in diabetic patients suggests that diabetes could affect magnesium homoeostasis, as previously discussed by some authors[6,21,22], especially when glycemic control is impaired[19], as observed in this study.

Another factor that may explain this observation is the increase in plasma insulin and insulin resistance in the diabetic group. This hormone stimulates magnesium transport from the extra- to the intracellular compartment, via Na+/H+ anti-transporter, favoring the passage of this mineral from plasma into the erythrocyte[22].

This insulin-stimulated modulatory mechanism could have contributed to the maintenance of magnesium levels in erythrocytes found in this study. Given the importance of magnesium in enzymatic processes, both at molecular and cellular levels, the body prioritizes the maintenance of intracellular concentrations of this mineral, and this compartment is reduced in chronic conditions[23].

The kidney plays a major role in magnesium homeostasis, increasing the resorption of this mineral when the body requires a greater demand or when dietary intake is l ow[12,24]. Thus, the reduced urinary excretion of magnesium seen in both groups was certainly due to lower intake of this mineral, as an attempt to maintain normal circulating levels of this mineral.

Plasma magnesium levels are very susceptible to changes because it is a central pool of exchange, and are directly affected by what is absorbed by the intestines and reabsorbed by the kid neys[12,24]. Despite the fine control exercised by the kidney, the lower magnesium levels found in the diabetic group in this study demonstrates that control of plasma magnesium in diabetes is more complex, and, additionally, may have influenced the lower creatinine clearance seen in the diabetic subjects.

Diabetic patients have reportedly reduced creatinine clearance, which is not always accompanied by increased serum and total magnesium, as occurs in non-diabetic subjects[20,25]. It was shown that in patients with type 2 diabetes, plasma magnesium levels are directly affected by creatinine clearance, and plasma magnesium can be reduced due to reduced clearance, at levels not indicating renal failure, which unable the compensation of magnesium levels based only on lower urinary excretion of this mineral and higher dietary intake of magnesium at levels similar to non-diabetic subjects[19], as observed in this study.

Table IV
Correlation between magnesium and iron biochemical parameters of subjects with type 2 diabetes and control groups

Parameters	Type 2 diabetes (n = 40)						Control (n = 48)					
	Serum iron (µg/dL)		Ferritin (µg/dL)		TIBIC (µg/dL)		Serum iron (µg/dL)		Ferritin (µg/dL)		TIBIC (µg/dL)	
	r	p	r	p	r	p	r	p	r	p	r	p
Plasma magnesium (mmol/L)	-0.139[a]	0.393	-0.138[a]	0.397	0.114[b]	0.482	0.392[a]	0.012	-0.063[a]	0.670	-0.100[a]	0.946
Erythrocyte magnesium (mmol/L)	-0.311[a]	0.049	-0.408[a]	0.009	-0.207[b]	0.200	0.089[a]	0.548	-0.153[a]	0.292	0.410[a]	0.782
Urine magnesium excretion (mmol/d)	-0.231[a]	0.151	-0.063[a]	0.697	-0.135[b]	0.405	-0.131[a]	0.386	-0.172[a]	0.254	-0.096[a]	0.537

[a] Pearson correlation. [b] Spearman correlation

It is worth noting that reduced plasma levels of magnesium in patients with type 2 diabetes may or may not be associated with increased urinary magnesium excretion, and the higher or lower excretion of this mineral will be determined by the presence of polyuria (urinary output >2,500 mL/d), resulting from hyperglycemia, as shown by Xu et al[26].

Given the reduced body levels of magnesium and the presence of poor glycemic control, in addition to higher percentage of glycation activity to glucose, it is expected a more likelihood to pro-oxidative state. The greater presence of TBARS in plasma of diabetic subjects in this study is an indication that this chronic hyperglycemia may have triggered glucose auto-oxidation, activation of protein kinase and overproduction of free radicals in the electron transport chain[27].

Likewise, hypomagnesemia may also be corroborating to the presence of lipid peroxidation, thereby increasing the presence of TBARS in diabetic subjects, as also observed by Guerrero-Romero and Rodríguez-Móran[28]. Magnesium deficiency promotes superoxide anion production and increased intracellular calcium concentration, which results in excessive production of nitric oxide and hydroxyl radical[29].

These factors, along with the presence of insulin resistance in type 2 diabetic patients, increased blood glucose, changes in lipid profile and endothelial dysfunction, promote oxidative damage in these patients, which may result in greater damage on pancreatic β cells. As these cells have lower concentrations of antioxidant enzymes, this contributes to increased production of free radicals[30].

In addition to this, there is concern regarding iron compartmentalization in this condition, since this mineral at high levels can be a pro-oxidant agent and, as a consequence of magnesium deficiency and diabetes, this mineral could have altered lev ls[5,7].

However, the concentrations of iron parameters assessed in this study were within the recommended normal values in both groups, and plasma levels of iron and ferritin were reduced, when compared to control group, in the diabetic subjects. Unlike magnesium, the dietary iron intake was considered appropriate in both groups. This observation of normal plasma levels of iron and ferritin in the diabetic subjects was also found by Zafar et al[31].

Inverse correlations between erythrocyte magnesium and plasma iron and ferritin in diabetic patients may indicate mechanisms triggered by the body to protect diabetic patients from increased iron levels and to prevent a more vulnerable oxidative state which could promote changes and damage. At normal levels, magnesium is described to have a protective effect on damage caused by iron overload, reducing hemolysis and the release of free iron[7].

Similarly, it has been reported that reduced erythrocyte magnesium levels are associated with increased iron in several t issues[7], and low dietary intake of magnesium in rats induces increased iron absorption and reduced number of red blood cells[5]. Given the magnesium deficiency, red blood cells would be more likely to have impaired structure and function, and thus, there is more likelihood for reduced levels of hemoglobin and red blood cell, which can simulate an anemia with increased levels of free iron[5,7].

One limitation must to be taken into consideration. The assessment of dietary intake is susceptible to random and systematic errors, and can be affected by the number of days. In order to minimize, the data of magnesium and iron obtained were subsequently adjusted on the basis of energy intake and intra-individual variation.

Conclusion

Given the above, it can be assumed that oxidative stress in diabetic patients appears to be due to factors involving diabetes pathophysiology, such as chronic hyperglycemia, and may also have been affected by altered magnesium levels, instead of being resulted from iron overload, since this mineral had appropriate levels to the parameters assessed in diabetic patients.

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