ALTERACIONES EN EL METABOLISMO DEL ZINC RELACIONADAS A LA EDAD

ALTERATIONS OF ZINC METABOLIC RELATED TO AGING

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ABSTRACT

Although many studies point to alterations in the organic concentrations of zinc in elderly patients, the mechanisms by which aging might cause changes in the metabolism of this nutrient remain unclear. Thus, we assessed the changes in plasma zinc, zinc binding capacity to plasma protein (ZnBCPP) and saturation index (SI), comparing elderly individuals and young adults. The zinc analyses were performed by atomic absorption spectrophotometry. A statistically significant difference ($p < 0.001$) was found between the two groups in relation to plasma zinc and SI, but the ZnBCPP did not differ between the younger and older subjects. In agreement with this result, it was shown in the young group that 76% ($R^2 = 0.760$) of the ZnBCPP variations are explained by the variations in plasma zinc and SI. In the elderly group this measure decreased to 30.5% ($R^2 = 0.305$). We conclude, therefore, that aging may be a factor associated to changes in control mechanisms and in zinc homeostasis, and could even alter ZnBCPP response patterns and other zinc-related indicators of nutritional status.
Key words: zinc, elderly, plasmatic protein, metabolism.

INTRODUCTION

Zinc is indispensable to the organism, due to the various biological functions it exercises in its role as a biochemical, regulating and structural agent. Even when there is a slight zinc deficiency, it is possible to detect consequences such as greater susceptibility to infection and cicatrization problems (1).

The elderly are at risk of zinc deficiency, due to their low dietary ingestion, lack of appetite, and interactions with medications. Moreover, there are indications that, although the zinc requirements of the elderly are similar to those of adulthood, the capacity to regulate the body pool is diminished (2).

Although there is much evidence that zinc deficiency, even a slight degree, is prevalent worldwide, particularly in specific population groups, such as the elderly, a totally reliable method to assess it has yet to be established (3).

Most studies, especially epidemiological, use plasma zinc as the measuring parameter. However, despite its convenience, this indicator is not considered to be sensitive enough to diagnose zinc status in the organism, since it is subject to circadian variations (4).

Therefore, more than one parameter must be used to perform this diagnosis. One of these parameters is Zinc Binding Capacity to Plasma Protein

In humans, serum zinc is nearly totally protein-bound, primarily to albumin and a_2-macroglobulin; and to a lesser extent to amino acids and transferrin. In healthy individuals it has been observed that the greater part of total serum zinc is bound to albumin and that only a small amount is bound to a_2- macroglobulin. This means that the measure of serum albumin and of total serum zinc can be used to estimate a physiologically important fraction of serum zinc in healthy patients (5).

In some situations, zinc binding to proteins is a result of the plasma zinc transfer to intracellular sites, which normally occurs after surgery or similar stressful events. Under more stable clinical conditions, nearly all the plasma zinc is bound to proteins, and changes in it concentration occur in response to factors unrelated to the availability of this metal (6).

The method that determines zinc-binding capacity in serum or plasma is based that a known amount of zinc ions saturate the protein carriers of this metal. Non-residual zinc or free zinc (not protein-bound) is determined by a reaction with magnesium hydroxicarbonate, which has a relatively high solubility. However, the product of this reaction, zinc hydroxide, has low solubility, precipitates immediately and, even if the reaction is displaced in the inverse direction, this compound continues to precipitate. This method ensures, however, that all the non protein-bound zinc is eliminated (or quelated) when there is excessive basic magnesium carbonate (7).

Some authors have reported that the Zinc Binding Capacity to Plasma Protein the only technique using serum is useful in determining moderate states of zinc deficiency and is more reliable that the simple measuring of zinc in these compartments (8– 10).

Thus, the aim of this study was to assess plasma zinc and ZnBCPP in adults and elderly individuals, to determine zinc-related changes in nutritional status caused by the aging process.

SUBJETCS AND METHOD

A cross-sectional study was conducted in a group of elderly subjects older than 60 years of age, of both sexes (n = 14); and the other group was composed of university undergraduates (n = 57) from the city of Natal, Brazil. The subjects were chosen randomly from those who declared themselves healthy and who had no history of chronic diseases and/or health problems in the previous year. The elderly underwent the Mini-Mental State Examination. The following exclusion criteria were established: the presence of gastrointestinal disorders, obesity or current malnutrition, chronic and/or acute diseases and the use of vitamin-mineral supplementation or other medication that might interfere in normal zinc metabolism. The study was approved by the Research Ethics Committee of the Federal University of Rio Grande do Norte (UFRN).

The zinc plasma dosage was calculated by atomic absorption spectrophotometry according to the method proposed by Rodriguez et al. (11), and the Zinc Binding Capacity to Plasma Protein (ZnBCPP) was determined using methodology standardized by Argemi et al. (10) and Cunningham et al. (8), modified by Pedrosa et al. (12).

Two 0.5 mL aliquots of plasma were removed and mixed in equal volume of a standard zinc solution of 10 mcg Zn/mL. The addition, in serum or in plasma, of a known amount of zinc ions, aimed at saturating the carrier proteins of this metal. The plasma proteins were then precipitated, with the addition of 85 mg of magnesium hydroxicarbonate and 4 mL of ultrapure water (MILLI-Q), which enabled the quantification of the free zinc (non protein-bound). After

centrifugation, the amount of zinc in the supernatant was analyzed by atomic absorption spectrophotometry (SPECTRA VARIAN AA-200). The results were expressed in µgzn/g of plasma protein.

The saturation index was calculated according to Arcasoy et al. (13), using the %SI formula (plasma Zn/ZnBCPP x 100).

The plasma proteins were measured colorimetrically with the BioSystems total protein kit.

To calculate Body Mass Index (BMI) we measured weight on a digital scale and height with a non-extendable tape measure.

Dietary zinc was assessed in the older group by a 3-day food record and in the younger group by a 24-hour record, and all data were subsequently analyzed with the Virtual Nutri program, version 1.0.

Student's t-test was used to analyze the independent samples and Pearson's correlation coefficient between the dependent variables was calculated considering a confidence interval of 95%. The linear regression model comprised backward selection between the plasma zinc variables, zinc binding capacity to plasma protein and saturation index, using SPSS software.

RESULTS AND DISCUSSION

Table 1 shows that the assessed groups had normal BMI and total protein values. The volunteers of this study, in general, had a good socioeconomic level, that is, they had access to health services and nutritional food. This situation is reflected in the concentration of plasma proteins, in which no statistical difference was found between the younger and older groups.

Plasma protein concentration is one of the parameters used to obtain ZnBCPP, whose alterations may influence this concentration. Since both groups had normal concentrations values with no significant difference between them, this result suggests that this variable had a low impact on the other results of the study.

TABLE 1
General characterization of the older and younger groups

Groups Variables	Older group (n=14)		Younger group (n=57)	
Age (years)	70.0 ($\pm$ 8.1)		22.8 ($\pm$ 3.3)	
Sex	Female	Male	Female	Male
	64.3%	35.7%	57.9%	42.1%
BMI[a] (kg/cm^2)	24.1 ($\pm$ 2.8)		22.3 ($\pm$ 2.6)	
Total Plasma Proteins[b] (g/dL)	7.5 ($\pm$ 0.78)		7.8 ($\pm$ 0.46)	

[a]Body Mass Index [b]Student's t-test (p= 0.161)

To assess the zinc x plasma protein relation, we performed a study that evaluated zinc distribution after acute myocardial infarction. An excellent correlation was observed between total zinc and protein-bound zinc, but practically no relation was found between zinc and its plasma carriers (albumin and a-2-macroglobulin) (14). The author suggests that the relation between zinc and plasma proteins is established by some additional factor and not only as a function of the amount of mineral in the organism and the presence of free carriers.

We suggest, therefore, that the aging process is one of the passive factors influencing this relation and the metabolic regulation of zinc.

Jong et al. (15) assessed a cohort of New Zealand women, whose plasma protein levels were normal, and found low plasma zinc values among the elderly and no association between this zinc parameter and the ingestion of the mineral. A similar situation was found by PEPERSACK et al (16), who evaluated elderly hospitalized patients, and also found no relation between low plasma zinc levels and the amount of plasma proteins. These results reinforce the need for other markers to better interpret and/or assess the zinc x plasma protein relation.

Table 2 shows the group differences in zinc concentration and in the saturation of its carriers, which seems to indicate changes in the metabolism of this mineral, possible related to age.

TABLE 2

Zinc related nutritional status variables of the younger and older groups

Variables	Older group (n=14)	Younger group (n=57)	
Plasma Zinc (ug Zn/dL)	82.86 (± 14.02)	104.19 (± 14.13)	**p < 0.001**
Saturation Index (%)	11.69 (± 2.06)	15.48 (± 3.30)	**p < 0.001**
ZnBCPP **(ug Zn/ g protein)**	96.04 (± 10.7)	88.88 (± 14.61)	p = 0.090

CI 95(%)

Although the plasma zinc values of the two groups were in the normal range, which is from 70 to 120 ug Zn/dL (17), there was a statistical difference between the older and younger subjects, finding also observed in plasma protein saturation, which was lower in the elderly. That is, there was less zinc bound to this group and therefore more free carriers, theoretically passive of being occupied by a larger amount of the mineral.

There was no statistical difference between the groups in relation to ZnBCPP, although it was higher in the elderly group. However, in the context formed by the other indicators, with plasma zinc and the saturation index differing statistically between the groups, a larger ZnBCPP value was expected than that found in the elderly subjects.

This being so, no proportional increase in ZnBCPP was found as a response to plasma zinc and the saturation index.

The dietary ingestion of zinc/day was 9.73 ± 0.91 mg in the younger group and 9.41 ± 1.0 in the older group, without statistically significant differences between them ($p = 0.8642$). Thus, we can deduce that the amount of dietary zinc has no effect on the differences found between the groups, in terms of plasma zinc and saturation index.

We can infer, therefore, that the older group had less "desire" and/or "competence" in binding zinc to the plasma protein pool, suggesting changes in zinc homeostasis. This could represent a greater difficulty in organic supply owing to a shortage and/or need to increase the continuous offer of zinc to reestablish this equilibrium.

The apparent change in the strength of this relation, where we can apparently relate aging with a smaller association between plasma protein saturation, plasma zinc and ZnBCPP, leads us to speculate about the differences between young adults and the elderly in dealing with high zinc doses, administered via supplements and/or their dietary effectiveness.

Grosshauer et al. (18) assessed ZnBCPP in athletes, before and after a zinc supplementation program. One of the groups evaluated showed significant differences in plasma zinc and ZnBCPP values, before and after supplementation; that is, as the amount of zinc increased, there was an increase in zinc plasma and an inversely proportional concomitant decrease in protein-binding capacity, related to the organic status of the mineral.

A study by Faure et al (19) analyzed zinc metabolism using stable isotopes, comparing a group of young women (mean age of 36 years) and a group of institutionalized (mean age of 73 years) and non-institutionalized (mean age of 72 years) elderly patients. Although zinc ingestion was similar in all the groups, differences were found in the metabolism of the mineral, in relation to the duration of the zinc in the plasma. The authors suggest that this may be related, among other factors, to the aging process and to some alteration in plasma protein-bound zinc.

Linear regression analysis showed that, in the younger group, the variations in ZnBCPP are explained in 76% ($R^2 = 0.760$) of the cases by plasma zinc variations and the saturation index. In the older group this response decreased to 30.5% ($R^2 = 0.305$). That is, there is a clear indication of changes in the mechanisms of zinc homeostasis related to aging, which involve alterations in the response patterns of ZnBCPP to the organic concentrations of zinc and their relation with the other zinc parameters in the organism.

Briefly, we observed this change in response pattern of the indicators, when we compared the older and younger groups, since, although the elderly apparently have more "space" to bind the zinc. This is reflected by a lower saturation index and lower amount of plasma zinc. This was also shown in the ZnBCPP, that is, although there is more space, the plasma proteins have seemingly less desire or ability to bind/capture zinc.

Mocchegiani et al (20) suggested that these changes in the binding of zinc to carrier proteins observed in aging may be considered potential markers to assess organism aging and a factor to be considered when defining proper diet for this stage of life.

Furthermore, Ravaglia et al. (21) and Savarino et al. (22) assessed groups at different life stages and found that, even among the elderly, there are differences between plasma zinc patterns, which seems to decline proportionally to age, being lower, for example, in 90 year-old individuals than in 70 year-old.

Mocchegiani et al. (23) offer evidence for understanding these mechanisms. According to these authors, over the course of the aging process there is an overexpression of some zinc-binding proteins such as metalloproteins and a-2-macroglobulin, which decreases zinc bioavailability in the organism. It is even speculated that this is one of the causes of immune function deficiency in the elderly.

We conclude therefore, that aging may be a factor associated to changes in control mechanisms and zinc homeostasis. It may even alter the response patterns of ZnBCPP and the other indicators of zinc-related nutritional status.

We suggest new studies, focused on the plasma protein profile, ZnBCPP and the response to different zinc supplementation regimens in the elderly, to help in the implementation of differential clinical practices for this vulnerable group.

RESUMEN

A pesar que muchos estudios indicarían que existen alteraciones en las concentraciones orgánicas del zinc en pacientes mayores, los mecanismos por los cuales el envejecimiento podría implicar cambios en el metabolismo de esta nutriente, aún permanecen pocos claros. Buscamos evaluar los cambios relativos al zinc plasmático, a la Capacidad de Ligación del Zinc a la Proteína Plasmática (ZnBCPP) y en el índice de Saturación (SI). Los análisis de zinc fueron realizados por espectrofotometría de absorción atómica, comparando personas mayores y adultos jóvenes. Una diferencia significativa fue encontrada ($p < 0,001$), entre los dos grupos, en relación al zinc plasmático e SI, siendo que la ZnBCPP no cambió entre los jóvenes y adultos mayores. Constatando este resultado, se demostró que en el grupo de jóvenes las variaciones en la ZnBCPP son explicadas en 76% ($R^2 = 0,760$) por las variaciones en el zinc plasmático en el SI. En el grupo de los mayores esta medida disminuye a 30,5% ($R^2 = 0,305$). Concluimos que el envejecimiento puede ser un factor asociado a los cambios en los mecanismos de control y homeostasis del zinc, alternando los patrones de respuesta relativos a ZnBCPP y algunos otros indicadores del estado nutricional relativo al zinc.

Palabras claves: zinc, envejecimiento, proteínas plasmáticas, metabolismo.

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