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Influence of cortisol on zinc metabolism in morbidly obese women

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Abstract

Introduction: **The accumulation of visceral fat affects the metabolism of hormones and some nutrients, but these mechanisms remain unclear.**

Objective: **To assess the influence of cortisol on the metabolism of zinc in morbidly obese women.**

Method: Cross-sectional, case-control study involving 80 women aged between 20 and 59 years. The participants were divided into two groups: experimental (morbidly obese, n = 40) and control (normal weight, n = 40). Zinc concentrations were determined by atomic absorption spectroscopy and serum and urinary cortisol by chemiluminescence method.

Results: **Zinc intake was significantly different between groups. Mean plasma zinc was lower in obese compared to control group. Mean values for erythrocyte zinc were 44.52 ± 7.84 μg/gHb and 40.17 ± 6.71 μg/gHb for obese and control groups, respectively. Urinary excretion of this mineral was higher in obese compared to control subjects (p < 0.05). Mean values for plasma cortisol were 9.58 ± 4.86 μg/dL for obese and 9.89 ± 5.61 μg/dL for control groups. Mean values for urinary cortisol were 163.00 ± 100.35 μg/dL and 109.71 ± 34.88 μg/dL for obese and control groups, respectively (p > 0.05). The correlation analysis between cortisol and zinc was not significant (p > 0.05).**

Conclusions: **Obese patients have hypozincemia and high erythrocyte zinc levels. The correlation between zinc parameters and cortisol concentration showed no influence of this hormone on zinc metabolism.**

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Key words: *Morbid obesity. Zinc. Glucocorticoids. Metallothionein.*

INFLUENCIA DEL CORTISOL SOBRE EL METABOLISMO DEL ZINC EN MUJERES OBESAS MÓRBIDAS

Resumen

Introducción: **El acúmulo de grasa visceral compromete el metabolismo de hormonas y de algunos nutrientes, sin embargo, esos mecanismos aún no están esclarecidos.**

Objetivos: **Evaluar la influencia del cortisol sobre el metabolismo del zinc en mujeres obesas mórbidas.**

Métodos: **Estudio transversal, caso-control, envolviendo 80 mujeres, en la faja etaria entre 20 y 59 años. Las participantes fueron distribuidas en dos grupos: experimental (obesas mórbidas, n = 40) y control (eutróficas, n = 40). Las concentraciones de zinc fueron determinadas por espectrofotometría de absorción atómica y el cortisol sérico y urinario, por quimioluminiscencia.**

Resultados: **La ingestión de zinc reveló diferencia significativa entre los grupos estudiados. Los valores medios de zinc plasmáticos fueron inferiores en las obesas cuando fueron comparadas al grupo control. El promedio de zinc eritrocítico fue de 44,52 ± 7,84 μg/gHb e de 40,17 ± 6,71 μg/gHb para las obesas y control, respectivamente. La excreción urinaria de este mineral fue superior en las obesas cuando comparadas al control (p < 0,05). Los valores medios del cortisol sérico fueron de 9,58 ± 4,86 μg/dL para las obesas y de 9,89 ± 5,61 μg/dL para el control. Las medias de cortisol urinario fueron de 163,00 ± 100,35 μg/dL e de 109,71 ± 34,88 μg/dL para obesas y control, respectivamente (p > 0,05). El análisis de correlación entre el cortisol y el zinc no fue significativo.**

Conclusiones: **las pacientes obesas presentan hypozincemia y elevada concentración de zin eritrocítico. La correlación entre los parámetros de zinc y las concentraciones de cortisol no demuestran influencia de esta hormona sobre el metabolismo del mineral.**

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Palabras clave: *Obesidad mórbida. Zinc. Glucocorticoide. Metalotioneína.*

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Abbreviations

HPA: Hypothalamic-Pituitary-Adrenal.
11β HSD-1: 11-β hydroxysteroid dehydrogenase type-1.
BMI: Body Mass Index.
WC: Waist Circumference.
EAR: Estimated Average Requirement.
MTF-1: Metal-Responsive Transcription Factor-1.
ZnT-1: Zinc Transporters 1.

Introduction

Adipose tissue is considered an endocrine organ that, when in excess, compromises the immune response, the metabolism of hormones and some nutrients; however, the mechanisms explaining how this tissue particularly acts on the bioavailability of minerals remain unclear.[1]

Recently, there has been a growing interest in regard to nutritional and hormonal disorders found in obese individuals aiming to elucidate the mechanisms involved in the pathogenesis of this disease. Regarding hormonal disorders, studies have shown changes in the metabolism of hormones, such as, cortisol. Accordingly, literature has evidenced changes in cortisol secretion, metabolism and sensitivity in obese individuals.[2,3]

With regard to this point, studies have shown that in visceral obesity, Hypothalamic-Pituitary-Adrenal (HPA) axis is hyper-responsive, and in obese patients, there is an increase both in the production and removal of this hormone, which contributes to sustain their plasma levels.[4]

An important factor to be noted is that, although the possible absence of biochemical changes in cortisol, disturbances in cortisol production are found in peripheral tissues, such as in adipose tissue and liver in the presence of obesity. Thus, adipose tissue, especially visceral fat, increases the expression of enzyme 11-β hydroxysteroid dehydrogenase type-1 (11β HSD-1), responsible to convert pre-receptor cortisone (inactive form) into cortisol (active form), contributing to increase the expression of this hormone.[4,5]

Regarding nutritional disorders found in obesity, studies have attempted to clarify the existence of changes in mineral metabolism. Some studies have shown that zinc concentrations in plasma and erythrocytes are reduced in obese women and zinc supplementation improves the physiological function.[6]

In parallel, literature has shown the association between disturbances in zinc concentrations and cortisol metabolism in obese subjects. This hormone stimulates the expression of metallothionein, a substrate that enhances cytoplasmic concentrations of this mineral by increasing the influx and release from intracellular organelles and thus indirectly increasing the intracellular concentrations of zinc and altering its bioavailability.[7,8]

Therefore, although some investigations have shown changes in zinc metabolism in obese patients, the mechanisms involved in this hypozincemia remain unclear. Thus, the determination of plasma and urine cortisol, as well as the relationship between these markers and zinc concentrations, contribute to clarify the influence of cortisol on zinc metabolism in obesity.

Methods

Cross-sectional, case-control study involving 80 female subjects aged between 20-59 years. The study participants were divided into two groups: experimental (morbidly obese, n = 40) and control (normal weight, n = 40). Obese women were spontaneously recruited from a private clinic.

Obese women were screened according to the following criteria: Body mass index (BMI) ≥ 40 kg/m^2, non-smokers, absence of chronic diseases, such as diabetes mellitus and chronic renal failure, liver disease or routine clinical inflammatory processes and also not using vitamin-mineral supplementation and/or use of other drugs that could affect the assessment of zinc-related nutritional status. The same criteria was applied in the control group, except that BMI of these subjects was 18.5-24.9 kg/m^2. The study was approved by the Research Ethics Committee of Federal University of Piauí.

Assessment of nutritional status

BMI of the participants was calculated dividing weight in kilograms by height in meters squared. Nutritional status was classified based on BMI and according to World Health Organization recommendations.[9]

For waist circumference (WC) a non-extensible measuring tape was used and the threshold values were according to WHO.[10]

Determination of zinc in diet

A three-day food record was used to assess food intake, and the analysis was performed using software Nutwin, version 1.5.[11] Estimated Average Requirement (EAR) was used employing 6.8 mg/day as the reference value for females.[12]

Determination of biochemical parameters for zinc

Samples of 15 mL were collected from the venous blood of fasting participants in the morning, between 7:30-8:30 a.m. Samples were placed in two tubes: one containing 30% sodium citrate as anticoagulant (10 μL/mL blood) for zinc analysis and another not containing anticoagulant for cortisol analysis.

Plasma was obtained by centrifuging whole blood at 1,831x*g* for 15 minutes at 4º C (CIENTEC® 4K15). Samples were collected using automatic pipette into demineralized polypropylene eppendorf tubes and stored at -20° C for later analysis.

To separate erythrocytes and further determine zinc, the method proposed by Whithehouse et al[13] was used. Red cell mass obtained from whole blood was washed with 5 mL 0.9% isotonic saline, and then slowly homogenized by inversion and centrifuged at 2493x*g* for 10 minutes at 4º C (CIENTEC® 4K15), and the supernatant was discarded. The procedure described was performed three times, to remove the contaminants from erythrocytes (platelets and leukocytes). After the last centrifugation, saline was aspirated, discarded and red cell mass was carefully extracted with the aid of an automatic pipette, transferred to demineralized polypropylene eppendorf tubes and maintained at -20° C for zinc and hemoglobin analysis.

Urine was collected over a period of 24 hours into demineralized plastic bottles with 5-L capacity, with no preservatives, using funnels also demineralized, which were provided to the study participants, securely sealed, labeled with the required instructions and kept refrigerated until the time to return to the researcher.

Zinc analysis in plasma, erythrocyte and urine was performed using flame atomic absorption spectrophotometry, according to the method described by Rodriguez et al[14]. Tritisol® (MERCK) prepared by dilution with Milli-Q® at concentrations of 0.1; 0.2; 0.3; 0.5; 1.0 µg/mL was used as standard.

Determination of cortisol

Serum was separated from whole blood by centrifugation at 1831x*g* for 15 minutes. Assessment of plasma cortisol levels was performed according to chemiluminescence method, and the standard reference range in the morning was 6-28.5 µg/dL.

24-hour urine was homogenized and urine volume was obtained using a 100-mL graduated cylinder. Then, one aliquot of the urine collected was collected into 40-mL plastic vials, with no preservatives and stored at -20º C for later analysis.

Free cortisol was determined in the urine following chemiluminescence method, using standard reference range of 28.5-213.7 µg/24 h.

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 $p < 0.05$. Spearman's correlation coefficient was used to check the potential interrelationship between variables.

Results

Mean and standard deviation values of the anthropometric parameters used to assess nutritional status of morbidly obese and control subjects are provided in table I.

Table II shows the mean and standard deviation values for zinc concentration in the diet, plasma, erythrocyte and urine of morbidly obese and control groups. A statistically significant difference was seen in the biochemical parameters related to zinc between groups ($p < 0.05$).

Mean and standard deviation values for serum and urinary cortisol levels of morbidly obese and control subjects are provided in table III. No statistically

Table I
Mean and standard deviation values for age, weight, height, body mass index and waist circumference of morbidly obese and control groups

Parameters	*Morbidly obese (n = 40) Mean ± SD*	*Control (n = 40) Mean ± SD*
Age (years)	32.33 ± 8.14	28.15 ± 6.83
Weight (kg)	113.77 ± 10.35*	57.3 ± 5.14
Height (cm)	1.61 ± 0.05	1.61 ± 0.61
BMI (kg/m^2)	43.73 ± 3.07*	22.18 ± 1.49
WC (cm)	114.63 ± 9.20*	72.08 ± 4.03

*Difference statistically significant between morbidly obese and control groups.
BMI = Body Mass Index; WC = Waist Circumference.

Table II
Mean and standard deviation values for zinc concentration in the diet, plasma, erythrocyte and urine of morbidly obese and control subjects

Parameters	*Morbidly obese Mean ± SD*	*Control Mean ± SD*	*p*
Dietary zinc (mg/day)$^{n=30}$	10.88 ± 4.25*	8.16 ± 2.66*	0.005
Plasma Zinc (μg/dL)$^{n=40}$	65.97± 12.30*	76.39 ± 13.18*	0.001
Erythrocyte Zinc (μg/gHb)$^{n=38}$	44.52 ± 7.84*	0.17 ± 6.71*	0.011
Urinary Zinc (μg/24 h)$^{n=31}$	564.75 ± 307.78*	355.02 ± 175.54*	0.002

*Difference statistically significant between morbidly obese and control groups, Student's t test ($p < 0.05$). Reference range for zinc in diet, plasma, erythrocyte and urine: EAR = 6.8 mg/day (female); 75-110 μg/dL; 40-44 μg/gHb; 300-600 μg Zn/24 h[12,15,16].

Table III
Mean and standard deviation values of serum and urinary cortisol concentrations of morbidly obese and control groups

Parameters	*Morbidly obese Mean ± SD*	*Control Mean ± SD*	*p*
Serum Cortisol$^{n=40}$ (μg/dL)	9.58 ± 4.86	9.89 ± 5.61	0.72
Urinary Cortisol$^{n=22}$ (μg/24 h)	163.00 ± 100.35	109.71 ± 34.88	0.096

No statistically significant difference was found between morbidly obese and control subjects, Mann-Whitney test ($p > 0.05$).
Reference values for serum and urinary cortisol: 6-28.5 μg/dL; 28.5-213.7 μg/24 h.

Table IV
Correlation between serum and urinary cortisol levels, anthropometric and biochemical parameters of zinc in morbidly obese patients

Parameters	*Serum cortisol (μg/dL)*		*Urinary cortisol (μg/24 h)*	
	R	*P*	*R*	*P*
BMI (kg/m^2)	0,137	0,401	0,019	0,932
WC (cm)	-0,229	0,155	-0,394	0,070
Plasma Zinc (μg/dL)	0.062	0.705	0.199	0.375
Erythrocyte Zinc (μg/gHb)	0.156	0.350	0.348	0.123
Urinary Zinc (μg/24 h)	0.127	0.496	0.325	0.140

There was no statistically significant difference between groups
BMI = Body Mass Index ; WC = Waist Circumference.

significant difference was found between groups regarding these parameters ($p > 0.05$).

The results of Spearman's correlation analysis between the parameters assessed are provided in table IV. No statistically significant difference was found.

Discussion

This study determined the biochemical parameters of zinc as well as serum and urinary concentrations of cortisol in morbidly obese women. Mean plasma zinc levels of obese subjects were lower than normal values, and the difference was statistically significant compared to the control group. These results are in agreement with those obtained by Ferro et al.[17] and Santos-Rocha et al.[18] who also found low plasma zinc levels in obese patients when compared with reference values.

It is important to emphasize that plasma is a parameter for zinc assessment as it maintains this mineral levels under constant homeostatic control, and may

indicate changes in their concentrations in response to stress, inflammation, action of hormones, such as glucocorticoids and food intake.[19] In this regard, we found that zinc content in the diet of obese patients was above the recommendations for this mineral, and this seems to not have affected the reduced plasma levels of this trace element found in the obese participants in this study.

Another factor pointed out in the literature as contributing to reduced plasma levels of zinc in obese patients is related to the actions of cortisol in the metabolism of this mineral. It should be noted that the presence of stress and chronic inflammation in obesity affects the synthesis of glucocorticoids, and this, in turn, induces the expression of metallothionein, a protein that promotes reduction in plasma zinc levels.[20]

An important point to be noted is regarding the distribution of zinc in the body, where 10-20% of this mineral in the blood is in the plasma and the remainder in the red blood cells. However, plasma zinc levels is the most used index to determine the zinc nutritional status. This parameter is valuable and useful to assess this mineral and the reduction indicates change in zinc homeostasis.[15]

Unlike the results obtained in plasma, zinc levels in erythrocytes were higher than normal in obese women of this study. These results are in agreement with those obtained by Pires et al.,[21] who also assessed morbidly obese patients and found a similar concentration of zinc in the red blood cells of these patients.

One factor that appears to favor increased erythrocyte zinc refers to the role of metallothionein as a regulator of the mineral homeostasis. Metallothioneins MT-I and MT-II are expressed in most cells, including erythrocytes. Thus, oxidative stress in obesity contributes to the release of zinc ions from this metalloprotein, which in turn increases the intracellular content.[22] On the other hand, most studies assessing metabolic changes of this mineral in obese patients have shown erythrocyte zinc levels below the reference range.[17,18]

Regarding zinc levels found in the urine of study participants, this was within normal range in both groups. However, the values found in obese women were significantly and statistically higher than control group. These results are in agreement with those found by Marreiro, Fisberg & Cozzolino,[23] who also found higher zinc levels in the urine of obese adolescents compared to control group.

An important aspect to be considered in this discussion concerns the body composition of the studied patients who are morbidly obese with BMI ≥ 40 kg/m^2 and waist circumference ≥ 88 cm, and this fact may have contributed to changes found in zinc metabolism. According to some researchers, these changes can be attributed to increased secretion of both cortisol and proinflammatory cytokines, who stimulate expression of proteins inducing changes in zinc metabolism.[24]

In this discussion, it is worth mentioning a study conducted in 1985 by Begin-Heick et al.[25] who found high zinc levels in the adipose tissue, liver and muscle of obese mice. These authors pointed out the influence of metallothionein on the influx of zinc in these tissues. Similarly, Bury et al.[7], found that cortisol could mediate the influx of zinc by activating metal-responsive transcription factor-1 (MTF-1), and thus inducing the gene expression of metallothionein and ZnT-1 (Zinc Transporters 1).

Correlation analysis between body composition parameters and serum cortisol concentrations in the experimental group tended to have a positive correlation between BMI and serum cortisol and negative correlation between WC and serum cortisol; however, these observations were not significant. It is important to note that BMI is an appropriate tool to identify trends in the prevalence of obesity, but does not consider the distribution of body fat. While WC measurement is a method allowing better identification of visceral fat accumulation.[26] Therefore, the negative correlation between WC and serum cortisol found in this study reinforces the influence of excessive visceral fat on the maintenance of normal levels of serum cortisol.

Accordingly, it is worth mentioning that visceral obesity is a cortisol disorder, and increased metabolic clearance and hyperesponsive HPA axis can contribute to the maintenance of normal levels of this hormone in the serum, since excessive body fat increases cortisol levels in the liver and adipose tissue.[2,3,27]

As for the cortisol levels found in this study, these were within normal range with no significant difference between groups. These results are in agreement with those found by Rask et al.[5] and Travison et al.[28] who also observed unchanged cortisol levels in obese patients when compared with individuals of normal weight.

With regard to urinary cortisol, the results were within the reference range in both groups, and no statistically significant difference was observed. These results are in agreement with those obtained by Vicennati et al.,[29] who assessed urinary free cortisol in healthy and obese subjects. It is important to highlight the advantage of determining free cortisol in the 24-hour urine, because this is an immune marker to fluctuations of cortisol binding protein, which is not affected by variations in the circadian rhythm.[30]

Increased urinary excretion of cortisol in obese patients is associated with high metabolic clearance found in these subjects and visceral fat accumulation.[31] Russell et al.[32] studied the urinary levels of cortisol as a marker of cardiovascular disease risk in obese adolescents and found significant increase of this hormone in the urine compared to the control group.

Regarding the results analyzing the relationship between biochemical parameters of zinc and serum and urinary cortisol levels in the experimental group, it was observed that there was no significant correlation. It

should be highlighted that cortisol acts indirectly on zinc metabolism, inducing gene expression of metallothionein and ZnT-1, which promotes the influx of this mineral, and therefore reduces their plasma levels.[7,20,33] This observation in association with normal levels of serum cortisol in the obese subjects could have contributed to the lack of correlation between these parameters assessed in this study.

Given the complexity of the mechanisms involved to alter the metabolic profile of minerals in obese patients, the different results found in the literature regarding this subject, as well as the potential influence of hormones and proinflammatory cytokines in these changes, new studies investigating the role of these molecules in zinc distribution can certainly contribute to better understand the metabolism of this mineral in obesity.

Conclusion

Food intake of zinc in both groups was high, and this was higher in morbidly obese patients. Regarding the biochemical parameters of zinc, obese women had reduced zinc concentrations in plasma, while this mineral was increased in red blood cells, maintaining the urinary values within normal range.

Morbidly obese women in this study did not show changes in serum and urinary cortisol levels and, thus, presence of hypercortisolemia and hypercortisoluria was not evidenced. Correlation between zinc levels in blood components and 24-hour urine and cortisol levels did not show influence of this hormone on zinc metabolism.

The results of this study lead us to suggest that abdominal fat accumulation, accompanied with increased visceral adipose tissue promotes changes in the metabolism of cortisol, which contributes to the increased expression of metallothionein and, consequent changes in zinc metabolism.

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