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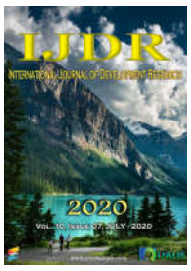

RESEARCH ARTICLE OPEN ACCESS

# NO ASSOCIATION BETWEEN CORTISOL, PARAMETERS OF ZINC, AND INSULIN RESISTANCE IN OBESE WOMEN

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**ABSTRACT**

**Objective**: Evaluate the association between cortisol, zinc parameters and insulin resistance in obese women. **Methods:** Case-control study was conducted, enrolling women aged between 20 and 50 years old, who were divided into case group (n=45) and control group (n=42). The dietary zinc intake was assessed by 3-day food records using Nut Win software version 1.5. Zinc concentrations in plasma, erythrocytes, and urine were determined by inductively coupled plasma optical emission spectrometry. Serum glucose and fasting insulin levels were determined by colorimetry and chemiluminescence, respectively. Serum cortisol concentrations and plasma glycated hemoglobin levels were determined by electro chemiluminescence and ion exchange chromatography, respectively. Insulin resistance was assessed by the HOMA-IR and HOMA2 indexes. Data were analyzed using the statistical software SPSS for Windows 20.0. **Results:** Serum cortisol concentrations did not present statistical difference between the groups ($p > 0.05$). Obese women had reduced plasma and erythrocyte zinc concentrations, when compared to the control group ($p < 0.05$).There was no statistically significant difference between the groups in the glucose and fasting insulin levels, and HOMA-IR and HOMA 2 indexes ($p > 0.05$). **Conclusions:** In addition, multiple linear regression analysis between serum cortisol, zinc parameters, and glycemic control parameters did not demonstrate the influence of this hormone on zinc metabolism and insulin resistance.



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## INTRODUCTION

Obesity is defined as excessive accumulation of body fat and can impair health and increase mortality (WHO, 2012). This disease alters the composition and structure of the adipose tissue and predicts important disorders, such as oxidative stress, inflammation, and endocrine and metabolic dysfunction (LOUWEN *et al*., 2018). Research has shown alterations in the metabolism of various hormones, such as cortisol, a glucocorticoid secretion and sensitivity with altered in obese subjects (GEER *et al*., 2014). Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis contributes to its hyperresponsiveness, with altered activity of the 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) enzyme and increased cortisol secretion, which is a risk factor for several metabolic disorders (MARTINS *et al*., 2014). Cortisol plays an important role in insulin resistance in obese individuals. This hormone acts as a functional antagonist of insulin and negatively

regulatesglucose uptake, as it releases energetic substrates for mitochondrial oxidation during stress, increasing muscle proteolysis, lipolysis of the adipose tissue and hepatic gluconeogenesis, impairing the glucose metabolism (HACKETT *et al*., 2016; KAMBLE *et al*., 2016). Importantly, cortisol induces the activation of the metal regulatory transcription factor 1 (MTF-1) and thereby up regulates the gene expression of metallothionein and Zip-14, leading to reduced plasma concentrations of zinc in obesity (TAKEDA *et al*., 2012; TAKEDA e TAMANO, 2010). Zinc plays a key role in the synthesis, storage and action of insulin by stimulating insulin receptors, protecting liver and pancreatic cells against free radicals. In addition, as a nutrient with an important function in insulin sensitivity, zinc participates in the stabilization of insulin hexamers (COOPER-CAPETINI *et al*., 2017; RANASINGHE *et al*., 2015). In this scenario, the objective of this study was to evaluate the association between cortisol, dietary zinc intake, zinc levels in plasma, erythrocytes and urine, and insulin resistance in obese women, as well as to evaluate food intake and parameters of glycemic control.

## MATERIALS AND METHODS

This was a case-control study enrolling women aged between 20 and 50 years old, who were divided into two groups: case group (women with a body mass index of 35 kg/m²; n = 45) and control group (women with a body mass index between 18.5 and 24.9 kg/m²; n = 42). Participants were selected after interview and met the following inclusion criteria: not being pregnant or nursing; no participation in another clinical study; no diagnosis of diabetes mellitus, chronic kidney disease, cancer and/or inflammatory bowel disease; no use of vitamin-mineral supplements and/or medicines that may interfere with the nutritional zinc status. Such information was self-reported by the participants. The study was protocolized and approved by the Research Ethics Committee of the Federal University of Piauí, under the opinion number 2.014.100, according to Resolution 466/12 of the Brazilian National Health Council (CNS). All participants signed a free and informed consent form (BRASIL, 2012).

**Nutritional Status Assessment:** To evaluate the nutritional status, the body mass index was calculated from the weight divided by theheight squared. Nutritional status classification was performed according to the recommendations of the World Health Organization (WHO).

**Measurements of zinc levels in plasma, erythrocytes, and urine:** A volume of 12 mL of venous blood was collected between 7 and 9 AM after 12 h fasting, and the blood amount was distributed among different tubes: (1) vacuum tube containing citrate for analysis of zinc and hemoglobin, (2) vacuum tube containing ethylenediaminetetraacetic acid (EDTA) for determination of glycated hemoglobin and (3) vacuum tube without anticoagulant for determination of serum glucose, insulin and cortisol. For plasma zinc measurement, plasma was separated from whole blood by centrifugation (CIENTEC® 4K15, São Paulo, Brazil) at 1831×g for 15 minutes at 4 °C. The plasma was aspirated with an automatic pipette, placed in polypropylene microtubes and stored at -20 °C. Erythrocyte separation was performed according to the methods proposed by Whitehouse *et al*. (1982). The erythrocyte mass was washed three times with 5 mL of isotonic saline (0.9% NaCl), carefully homogenized by inversion and centrifuged (CIENTEC® 4K15, São Paulo, Brazil) at 2493 × g for 10 minutes, and the supernatant was aspirated and discarded. After the last centrifugation, the saline solution was discarded, and the erythrocyte mass was carefully aspirated with an automatic pipette and transferred to microtubes, which were stored at -20 °C for measurement of zinc levels. The described procedure was performed three times to remove any contaminants from erythrocytes (i.e., platelets and leukocytes). For 24-hour urine collection, demineralized containers were weighed before and after collection on a semi-analytic scale, for determination of urinary volume from the density. After this procedure, 20 mL of urine was removed, distributed among polypropylene microtubes and stored at -20 °C for later measurement of zinc levels. Measurement of the zinc concentration in the samples was performed 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, United States). The reference values adopted were 75-110 µg/dL for plasma zinc levels (GIBSON, 2005), 40-44 µg/gHb for erythrocyte zinc levels (GUTHRIE e PICCIANO, 1994) and 300-600 µg/24 hours for urinary zinc levels (GIBSON, 2005).

**Hemoglobin concentration:** Hemoglobin concentration in the erythrocyte mass was determined according to the cyanmethemoglobin method to express erythrocyte zinc concentrations. The absorbance wasread on a visible UV spectrophotometer (Bel Photonics®, SP1102, Brazil) using the wavelength of 540 nm.

**Determination of Serum Cortisol:** The serum cortisol concentration was always measured in the morning, and the reference values were within 6.23-18.01 µg/dL (NIEMAN *et al*., 2008).

**Determination of Glycemic Control:** Measurement of fasting glucose was performed by the colorimetric enzymatic method using Labtest kits. Values between 75 and 99 mg/dl were considered normal, according to the criteria defined by the American Diabetes Association (ADA, 2017). Serum insulin concentration was measured by chemiluminescence method, and values between 6 and 27 µU/ml were considered normal. Insulin resistance was determined using the Homeostasis Model Assessment Insulin Resistance (HOMA-IR1) index, which was calculated from the concentrations of fasting glucose and fasting insulin. The HOMA-IR2 was calculated using the HOMA Calculator version 2.2.2 (HOMA CALCULATOR, 2017). Measurement of glycated hemoglobin was made using the ion exchange chromatography method. Values between 5.7 and 6.4% indicated a high risk of diabetes (ADA, 2017).

**Statistical analysis:** Data were analyzed using SPSS software for Windows® version 20.0. Data distribution was assessed by the Kolmogorov-Smirnov test. For comparison between groups, Student's t-test was used for data with parametric distribution, and the Mann-Whitney test was used for data with non-parametric distribution. The tests were used to compare the means of plasma, erythrocyte and urinary zinc among the three groups according to the body mass index: eutrophic women (body mass index between 18.5 and 24.9 kg / m²), women with obesity level II (body mass index between 35 and 39.9 kg / m²) and level III (body mass index ≥ 40 kg / m²). The analysis of variance (ANOVA) was used for comparisons among the groups, considering the parametric distribution of the data. The Tukey and Bonferroni tests were used to compare the means among different treatments. To test for correlations, Pearson's linear correlation coefficient was used for data with normal distribution. Associations between the variables were calculated using the Chi-square test and the degree of association was tested using the Cramer's coefficient. The difference was considered statistically significant when the p value was lower than 0.05, adopting a confidence interval of 95%.

## RESULTS

The mean values and standard deviations of age and anthropometric parameters used in the assessment of the nutritional status are presented in Table 1. Statistical difference was observed in all anthropometric parameters ($p < 0.05$).

**Table 1. Mean values and standard deviations of age, bodyweight, height, body mass index and waist circumference of the control group and obese participants.**

| Parameters | Control (n = 45) Mean ± SD | Obese (n = 42) Mean ± SD | p |
|---|---|---|---|
| Age (years) | 34.9 ± 7.9 | 32.2 ± 8.3 | 0.255 |
| Bodyweight (kg) | 55.9 ± 5.9 | 107.8 ± 14.5* | <0.001 |
| Height (m) | 1.6 ± 0.1 | 1.6 ± 0.1* | 0.010 |
| BMI (kg/m$^2$) | 22.6 ± 1.7 | 41.6 ± 5.6* | <0.001 |
| WC (cm) | 74.4 ± 5.0 | 115.1 ± 12.1* | <0.001 |

*Significantly different values between obese patients and control group, Student's t-test or Mann-Whitney test (p<0,05). BMI = body mass index; WC = waist circumference

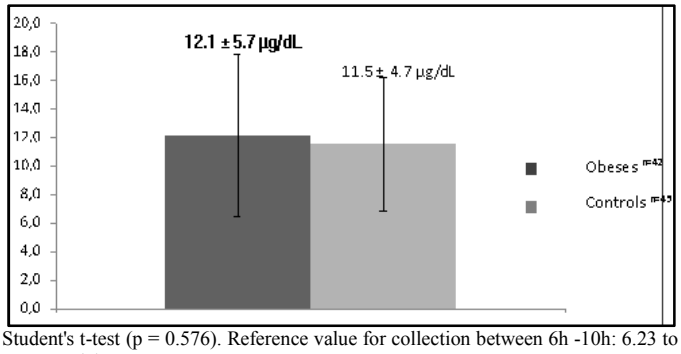
The mean values and standard deviations of energy intake and dietary intake of zinc and other micronutrients are described in Table 2. No significant statistical difference was observed between the groups regarding energy intake and dietary amounts of carbohydrates, proteins, lipids and zinc.

**Table 2. Mean values and standard deviations of energy in take, macronutrientsandzinc from the control group and obese participants**

| Parameters | Control (n = 39) Mean ± SD | Obese (n = 25) Mean ± SD | p |
|---|---|---|---|
| Energy (Kcal/day) | 1707.1 ± 357.2 | 1591.1 ± 489.6 | 0.278 |
| Carbohydrates (%) | 51.7 ± 6.4 | 50.2 ± 9.9 | 0.494 |
| Proteins (%) | 28.8 ± 4.2 | 29.2 ± 5.1 | 0.398 |
| Lipíds (%) | 19.5 ± 3.3 | 20.5 ± 5.9 | 0.724 |
| Dietar y zinc (mg/day) | 11.6 ± 2.1 | 10.6 ± 4.1 | 0.284 |

Student's t-test (p> 0.05). Reference Values: 45 to 65% carbohydrate, 10 to 35% protein, and 20 to 35% lipid; EAR = 6.8 mg zinc / day, age range between 19 and 50 years (female).

Figure 1 shows the serum cortisol concentrations of obese participants and control group. No significant statistical difference was observed between the groups (p = 0.576).




Student's t-test (p = 0.576). Reference value for collection between 6h -10h: 6.23 to 18.01 µg / dL.

**Figure 1. Mean values and standard deviations of serum cortisol concentrations (µg / dL) of obese participants and control group**

Table 3 shows zinc concentrations in plasma, erythrocyte and urine in the control group and in obese participants. Statistically significant differencein all these levelswas observed between the groups (p <0.05).

**Table 3. Mean values and standard deviations of plasma, erythrocyteandurinaryzinc concentrations of the control group and obese participants**

| Parameters | Control (n = 45) Mean ± SD | Obese (n = 42) Mean ± SD | P |
|---|---|---|---|
| Plasma zinc (µg/dL) | 89.5 ± 12.4 | 67.3 ± 6.4* | <0.001 |
| Erythrocytezinc (µgZn/gHb) | 42.7 ± 3.6 | 37.2 ± 3.7* | <0.001 |
| Urinaryzinc (µg/24h)[#] | 208,9 ± 94,9 | 293,4 ± 108,8* | <0.001 |

* Significantly different values between obese patients and control group, Student t test (p <0.05). Reference values: Erythrocyte zinc = 40 to 44 µg / gHb(GUTHRIE e PICCIANO, 1994); Plasma zinc = 75-110 µg / dL(GIBSON, 2005).[#]Urinary zinc: control n=43,obese n=28.

The mean values and standard deviations of the glycemic control parameters in the control group and in obese participants are shown in Table 4. A statistically significant difference was found in glycated hemoglobinlevels between the groups (p = 0.022).

**Table 4. Mean values and standard deviations of glycemic control parameters of the control group and obese participants.**

| Parameters | Control (n = 45) Mean ± SD | Obese (n = 42) Mean ± SD | p |
|---|---|---|---|
| Glucose (mg/dL) | 80.7 ± 8.3 | 84.7 ± 12.9 | 0.089 |
| Insulin (µU/mL) | 10.0 ± 2.5 | 10.7 ± 3.6 | 0.312 |
| HbA1 (%) | 5.0 ± 0.5 | 5.2 ± 0.5* | 0.022 |
| HOMA-IR | 2.0 ± 0.6 | 2.2 ± 1.0 | 0.133 |
| HOMA2-IR | 1.3 ± 0.3 | 1.3 ± 0.5 | 0.227 |

* Significantly different values between obese patients and control group, Student t test (p <0.05). HbA1 = glycatedhemoglob in; HOMA-IR = Homeostasis Model Assessment Insulin Resistance; HOMA2-IR = Homeostasis Model Assessment. Reference values: Fasting Glucose = 75 to 99 mg / dL; Seruminsul in = 6 to 27 µU / mL; HbA1 <5.7; HOMA-IR> 2.71; HOMA2-IR> 1.8.

Table 5 shows the results of the correlation analysesbetween levels of zinc, cortisol and markers of glycemic control in obese participants. No significant correlation between variables was identified (p> 0.05).

**Table 5. Simple linear correlation analysis between zinc, cortisol and glycemic control parameters in obese patients**

| Parameters | ZincDietary | | Plasma Zinc | | ErythrocyteZinc | |
|---|---|---|---|---|---|---|
| | r | p | r | p | r | p |
| Cortisol | -0.035 | 0.969 | 0.105 | 0.509 | 0.058 | 0.713 |
| Glucose | 0.077 | 0.713 | -0.151 | 0.341 | 0.001 | 0.998 |
| Insulin | 0.156 | 0.455 | -0.131 | 0.409 | -0.014 | 0.931 |
| HbA1 | -0.004 | 0.984 | -0.229 | 0.145 | -0.023 | 0.887 |
| HOMA-IR | 0.162 | 0.440 | -0.173 | 0.272 | -0.033 | 0.837 |
| HOMA2-IR | 0.159 | 0.448 | -0.152 | 0.341 | -0.020 | 0.900 |

HbA1 = glycatedhemoglobin; HOMA-IR = Homeostasis Model Assessment Insulin Resistance; HOMA2-IR = Homeostasis Model Assessment; Plasma zinc (ug / dL); Erythrocytezinc (µg / gHb); Urinaryzinc (µg / 24 hours); Glucose (mg / dL); Insulin (µU / mL).

The correlation analysis between serum cortisol and glycemic control parameters of both groups did not show a statistically significant result (p> 0.05). Multiple linear regression analysis showed that plasma zinc, erythrocyte, urine serum and cortisol were not predictive of the glycemic parameters in both groups.

## DISCUSSION

We evaluated the association between serum cortisol, zinc biomarkers and insulin resistance in obese women. Cortisol serum concentrations of obese participants were adequate and no statistically significant difference was observed in these levels when compared to the control group. Some factors may explain the adequate serum cortisol concentrations in obese women, such as the fact that increased serum levels of this hormone are able to inhibit thesecretion of corticotropin releasing hormone (CRH) and adrenocorticotropin (ACTH), which induce cortisol secretion (GATHERCOLE *et al*., 2011). This negative feedback mechanism favors the maintenance of serum cortisol concentrations within the normal range.

It is noteworthy that, although hypercortisolemia has not been verified in obese participants in this study, it may be assumed that the conversion of this hormone into its biologically active form occurs in target tissues, which allows its expressive performance, even under adequate concentrations (SVENDSEN *et al*., 2009), according to the results of this

study. The dietary zinc intake was found to be higher than recommended in obese patients. These results are in accordance with the reports of Cominetti *et al*. (2006); Ferro *et al*. (2011); and Martins *et al*. (2014). The high zinc intake by the participants of this research can be explained by the food habitsin the Brazilian population, characterized by consumption of foods rich in proteins, mainly red meat and other foods of animal origin, which are sources of this mineral (GIBSON, 2012; IBGE, 2011). Obese women presented plasma levels zinc below the normal range, with a statistically significant difference, when compared to the control group. These data are in accordance with the findings of Samad *et al*. (2017) and Suliburska *et al*. (2013). The zinc concentration in erythrocytes of obese women was shown to be significantly reduced, when compared to the control group, and below the standard of normality. This result reflects chronic changes in the zinc nutritional status, aserythrocytes have a long half-life (120 days), which shows the presence of disturbances in the long-term zinc homeostasis in obese women. Considering the possible effects of cortisol on zinc homeostasis, how to induce the expression of metallothionein and ZIP-14 and reduce blood zinc, a correlation analysis between these two variables was conducted. However, no correlation was found between serum cortisol concentrations and zinc parameters.

Of note, serum cortisol concentration does not reflect cortisol metabolism, i.e., it does not allow the identification of how much cortisol is secreted and converted into its biologically active form. Serum cortisol measurement alone may have limited the achievement of a more consistent result regarding its influence on zinc metabolism, considering that the effects of cortisol can be amplified in specific tissues irrespective of its circulating concentrations. We also evaluated the glycemic control of the study participants. Serum glucose and insulin levels and mean values of the insulin resistance index were within the normal range and were not significantly different between the groups. However, glycated hemoglobin was significantly higher in the case groupthan in the control group, even within normal values. No correlation was found between the biochemical markers of zinc status and glycemic control in the obese participants. Multiple linear regression analysis found that neitherbiochemical markers of zinc statusnor serum cortisol concentration were predictive of the glycemic parameters in both groups. Some factors that may have contributed to the absence of significant results, such as adequate cortisol serum concentrations and absence of peripheral insulin resistance. It is worth mentioning that the analysis of other markers of cortisol metabolism, such as cortisol in saliva and urine, as well as possible errors of measurement, transport and handling of the samples may not constitute limitations for a more in-depth discussion of the results. In addition, our sample size may have prevented statistically significant results, particularly regarding food consumption data. We have the prospect of advances in the evaluation of other cortisol biomarkers that can provide a more effective response on the performance of this hormone in obese organisms. Moreover, the interaction between cortisol and mechanisms involved in the zinc metabolism must be further assessed by molecular approaches.

**Conclusion**

Obese women evaluated in this study had adequate serum cortisol concentrations and did not show insulin resistance. An inadequate zinc nutritional status was also observed, characterized by high dietary values, with reduced concentrations in erythrocytes, plasma and urine. In addition, no evidence of cortisol influence on the zinc metabolism and insulin resistance was found in obese women.

**ListofAbbreviations**

| | |
|---|---|
| 11β-HSD1 | 11β-Hydroxysteroid Dehydrogenase 1 |
| ACTH | Adrenocorticotropin |
| ANOVA | Analysis of Variance |
| BMI | Body Mass Index |
| CNS | Brazilian National Health Council |
| CRH | Corticotropin Releasing Hormone |
| EDTA | Ethylenediaminetetraacetic |
| HbA1 | GlycatedHemoglobina |
| HOMA-IR1 | Homeostasis Model Assessment Insulin Resistance |
| HOMA2-IR | HomeostasisModelAssessment |
| HPA | Hypothalamic-Pituitary-Adrenal |
| MTF-1 | Regulatory Transcription Factor 1 |
| WC | WaistCircumference |
| WHO | World Health Organization |

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