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COPPER NUTRITIONAL STATUS AND ITS RELATIONSHIPS WITH OXIDATIVE STRESS IN OBESE WOMEN

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ABSTRACT

Background and Aims: Obesity is a disease that is characterized by the excessive accumulation of adipose tissue that favors the development of metabolic disorders such as those related to oxidative stress and appears to contribute to changes in the homeostasis of micronutrients involved in the production of free radicals such as copper. From this perspective, there is an interest in regard to identifying the possible influence of this mineral on the manifestation of oxidative stress in the context of obesity. Materials and methods: A total of 141 women were divided into the case group (BMI≥ 35 kg/m^2) and the control group (BMI between 18.5 and 24.9 kg/m^2). Weight and height were measured, and BMI values were calculated. Dietary copper intake was obtained from a standard 3 day food record. Analyses of copper in plasma and erythrocytes were performed, and the concentrations of the enzymes superoxide dismutase, glutathione peroxidase, and catalase were determined. Additionally, serum concentrations of thiobarbituric acid-reactive substances were determined. Results: The amount of copper observed in the diets of obese women was higher than was that in the control group. Elevated copper concentrations in the plasma and a reduction in erythrocytes were observed, and high TBARS values and reduced activity of the erythrocyte superoxide dismutase enzyme were also observed in the case group compared to these values in the control group. Conclusion: Our study did not identify a correlation between the assessment parameters of nutritional status related to copper and the concentrations of TBARS in either group.

Key words: Copper. Micronutrients. Oxidative Stress. Adipose Tissue. Obesity.

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RESUMO

Estado nutricional do cobre e suas relações com o estresse oxidativo em mulheres obesas

Justificativa e objetivos: A obesidade é uma doença que se caracteriza pelo acúmulo excessivo de tecido adiposo que favorece o desenvolvimento de distúrbios metabólicos como os relacionados ao estresse oxidativo e parece contribuir para alterações na homeostase de micronutrientes envolvidos na produção de radicais livres como o cobre. Nesta perspetiva, existe um interesse em identificar a possível influência deste mineral na manifestação do stress oxidativo no contexto da obesidade. Materiais e métodos: Um total de 141 mulheres foi dividido no grupo caso (IMC≥ 35 kg/m^2) e no grupo controlo (IMC entre 18,5 e 24,9 kg/m^2). O peso e a altura foram medidos, e os valores de IMC foram calculados. A ingestão de cobre na dieta foi obtida a partir de um registo alimentar padrão de 3 dias. Foram efectuadas análises de cobre no plasma e nos eritrócitos, e foram determinadas as concentrações das enzimas superóxido dismutase, glutationa peroxidase e catalase. Adicionalmente, foram determinadas as concentrações séricas de substâncias reactivas ao ácido tiobarbitúrico. Resultados: A quantidade de cobre observada nas dietas das mulheres obesas foi superior à do grupo de controlo. Foram observadas concentrações elevadas de cobre no plasma e uma redução nos eritrócitos, bem como valores elevados de TBARS e uma redução da atividade da enzima superóxido dismutase eritrocitária no grupo de casos, em comparação com os valores do grupo de controlo. Conclusão: Nosso estudo não identificou correlação entre os parâmetros de avaliação do estado nutricional relacionados ao cobre e as concentrações de TBARS em nenhum dos grupos.

Palavras-chave: Cobre. Micronutrientes. Stress oxidativo. Tecido adiposo. Obesidade.

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INTRODUCTION

Obesity is characterized by excessive accumulation of body adipose tissue and has a complex and multifactorial etiology involving genetic, environmental, and nutritional factors.

This disease exerts an important impact on public health, seems contributes to the manifestation of other chronic diseases such as type 2 diabetes mellitus, non-alcoholic fatty liver disease, cardiovascular disease, and cancer (Habib e colaboradores, 2015; Marseglia e colaboradores, 2014; Ortega e colaboradores 2012).

Adipose tissue dysfunction is characteristic of obesity and results from a prolonged positive energy balance and the accumulation of hypertrophied adipocytes, particularly in the abdominal region. This is an important factor for the development of metabolic, hormonal, and molecular disorders such as those related to oxidative stress that is characterized by excessive production of reactive oxygen species (ROS) associated with reduced antioxidant defense (Goossens, 2017; Longo e colaboradores, 2019; Tureck e colaboradores, 2017, Valko e colaboradores 2007; Keane e colaboradores, 2015).

Oxidative stress directly or indirectly alters vitamin and minerals metabolism that either directly or indirectly act on the antioxidant defense system (Banache e colaboradores, 2020; Manna, Jain, 2015; Morais e colaboradores, 2017).

Within this context, the literature has reported changes in copper concentrations, particularly in individuals with excess adipose tissue (Feldman e colaboradores, 2015; Gu e colaboradores, 2019; Yang e colaboradores, 2019).

It is noteworthy that copper plays numerous roles in energy, glucose, and lipid metabolism in addition to acting in important biochemical reactions in the nervous system and in antioxidant defense, and this micronutrient is essential for the biosynthesis of the enzyme superoxide dismutase. However, the results of certain studies reveal that this mineral when present in high amounts can play a pro-oxidant role through the Fenton and Haber-Weiss reactions and consequently favors the manifestation of oxidative stress (Fan, Zhang e Bu, 2017; Tinkov e colaboradores, 2012; Valko e colaboradores, 2005).

Therefore, given the scarcity of data detailing changes in copper metabolism in obese individuals, this study aimed to obtain information regarding the copper status in obese women and it's the relationship between this mineral and markers of oxidative stress.

MATERIALS AND METHODS

Study characterization and experimental protocol

This study used a cross-sectional design and a convenience sample of 202 adult women who were distributed into two groups: case group (obese women, n=62) and control group (eutrophic women, n=79). This is a hospital-based study, and the participants were recruited at an outpatient clinic through verbal invitation while waiting for the medical appointment.

The outpatient clinic is located in the city of Teresina, in the state of Piauí, Brazil.

Participants were selected through an interview, according to previously established inclusion criteria: age between 20 and 50 years; body mass index (BMI) from 30.0 kg/m^2 or higher (case group), and between 18.5 and 24.9 kg/m^2 (control group); absence of diabetes, chronic kidney disease, cardiovascular disease, liver disease, inflammatory bowel disease, cancer, or recent infections; not pregnant or breastfeeding; not participating in another clinical study; not using any vitamin-mineral supplement and/or medications that may interfere with copper metabolism; non-smokers; no chronic alcohol intake.

The project was registered in the Brazilian platform, and it was approved by the Research Ethics Committee of the Federal University of Piauí, according to the CNS Resolution 466/12, under Certificate of presentation for Ethics Appreciation (CAAE) Nº. 3.276.485. All participants signed an informed consent form describing the study.

Evaluation of anthropometric parameters

Body weight, height and waist circumference of the participants of the study and control groups were measured in triplicate, according to the methodology described by the Ministry of Health. BMI was calculated as the ratio of the participant's weight in kilograms divided by the height in meters squared (BMI =

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kg/m²) (IBGE, 2011; Nolasco, 1995; WHO, 2000).

Dietary intake assessment

We have used three-day food record method to analyze food intake. We have estimated dietary intake of the energy, macronutrients, and copper using the software "NutWin", version 1.5 (Sao Paulo, Brazil) (Anção e colaboradores, 2002). Food information that was not available in the software was included in the software from the Brazilian Table of Food Composition (TACO, 2011) and the Food Composition Table of the Brazilian Institute of Geography and Statistics (IBGE) (IBGE, 2011).

We have estimated the usual dietary intake of copper and macronutrients with Multiple Source Method - MSM by a logistic regression model. This program can be accessed through the MSM website that can be reached at https://nugo.dife.de/msm/. Dietary intake data of copper and macronutrients also were energy-adjusted by the residual method, reducing the influence of differences in energy consumption on the dietary copper and macronutrients intake. Dietary intake data were energy-adjusted by nutrient calculation after verifying the normality of the data distribution [Fisberg e colaboradores, 2005; Jaime e colaboradores, 2003; Willett, Stampfer, 1986).

Collection and processing of biological material

We have collected samples of 8 mL of venous blood samples in the morning, between 7 and 9 am, and the participants have been fasting for at least 12 hours. The collected blood was distributed in EDTA tubes for determination of copper, thiobarbituric acid reactive substances, and for determination of antioxidant enzyme activity in the different blood compartments.

Plasma was separated from whole blood by centrifugation (CIENTEC® 4K15, São Paulo, Brazil) at 1831 x g for 15 minutes at 4 ºC, and then extracted with an automatic pipette and stored in polypropylene microtubes. Then, the samples were preserved at -20 °C (for copper determination). Method proposed by Whitehouse colaboradores (1982) was used for red cell separation and subsequent copper determination. The erythrocyte mass was washed with 10 mL of isotonic saline solution (NaCl 0.9%), being carefully mixed by inversion and then centrifuged (SIGMA® 4K15) at 2493 x g for 10 minutes.

This procedure was repeated three times to remove contaminants from the erythrocytes (platelets and leukocytes). After the last centrifugation, the saline solution was aspirated and discarded, and the erythrocyte mass was carefully extracted with the aid of an automatic pipette. Subsequently it was transferred to demineralized polypropylene tubes, kept at -20°C for future analysis (Harrington e colaboradores, 2014).

Determination of plasma and erythrocyte copper

Plasma and erythrocyte copper concentrations were determined by inductively coupled plasma optical emission spectrometry (720 ICP/OES, Varian Inc., United States) Harrington e colaboradores, 2014; Niedzielski, Siepak, 2003. The apparatus was configured with the following experimental conditions: Power: 1.4 kW; Plasma flow (gas): 15 L/min; Auxiliary gas flow: 1.5 L/min; Type of spray chamber: cyclonic; and a nebulizer gas flow rate of 0.7 L min −1.

Plasma samples were diluted 1:20 (v/v) as follows: 3.0% (w/v) 1-butanol, 0.1% (v/v) N-Methylaniline trifluoroacetate (Sigma Aldrich nº 210080 Merck KGaA, Darmstadt, DE), 0.05% (v/v) HNO3. Red blood cells/RBC samples were diluted 1:60 (v/v) as follows: 3.0% (w/v) 1-butanol, 0.2% (v/v) N-Methylaniline trifluoroacetate (Sigma Aldrich nº 210080 Merck KGaA, Darmstadt, DE), 0.1% (v/v) HNO3. All standards were prepared in the same way as the samples. Calibration curves were prepared at the following concentrations: 1, 5, 10, 20, 50 and 100 µg/L in diluent solutions containing 3.0% (w/v) 1-butanol, 0.1% (v/v) N-Methylaniline trifluoroacetate (Sigma Aldrich nº 210080 Merck KGaA, Darmstadt, DE) and 0.05% (v/v) HNO3. The relative recoveries for the proposed method ranged between 99% to plasma and 85% to erythrocyte samples, while the relative standard deviation values were lower than 9% for each, suggesting good accuracy and precision for this method.

The choice of spectral lines for analysis was based on both their sensitivity and spectral interference. The reading for copper was performed at the wavelength of 324.760 nm. Detection limits were determined from the equation: 3 × standard deviation of 10

measurements of the blank, divided by the slope of the calibration curve. Samples of certified reference material (Seronorm® OligoelementSerum, Norway) were determined to validate the analytical measurements in ICP-OES. Reference values were considered between 80 and 155 µg/dL for plasma copper (Burtis, Ashwood, 1998); and 30.5 - 132.2 µg/dL for erythrocyte copper (Vitoux e colaboradores, 1999).

Determination of markers of oxidative stress

The activity of glutathione peroxidase and superoxide dismutase enzymes in erythrocytes was evaluated in an automatic biochemical analyzer (Labmax 240 model, Lagoa Santa, Mg, Brazil); using the Ransel 505 kit (Randox Laboratory, Crumlin, UK). And the activity of catalase enzime was determined in Bel Photonics UV/Vis spectrophotometer (model SF200DM, Osasco, SP, Brazil) at 240 nm, according to the method proposed by Aebi (Aebi, 1984).

The determination of plasma concentrations of Thiobarbituric Acid Reactive Substances (TBARS) was performed according to the method proposed by Ohkawa, Ohishi; Yagi (1979). The calibration curve was prepared using concentrations of 0.5; 1.0; 2.0; 4.0 and 8.0 nmol/mL of the standard reagent 1,1,3,3-tetraethoxypropane (Sigma-Aldrich®).

Statistical analyses

Data were organized in Microsoft Excel® spreadsheets for descriptive analysis of the variables observed in the groups studied. Later, they were exported to the SPSS program (for Windows® version 26.0) for statistical analysis of the results.

The Kolmogorov-Smirnov test was applied to verify the normality of the data. Then, for comparison purposes between the groups studied, the Student's t-test was used for variables with normal distribution, and the Mann-Whitney U-test for those with non-parametric distribution.

Test was performed to compare the means of the variables dietary, plasma and erythrocyte copper, and also the markers superoxide dismutase, catalase, glutathione peroxidase and TBARs among three groups distributed according to BMI: control group (women with body mass index between 18.5 and 24.9 kg/m^2), group with grade II obesity (body mass index between 35 and 39.9 kg/m^2), and with grade III obesity (body mass index ≥40 kg/m^2). To do so, analysis of variance (ANOVA) or the Kruskal-Wallis test was used for variables with parametric/ homogeneous and nonparametric/ nonhomogeneous distributions, respectively. Bonferroni and Tukey post-hoc tests were used. All tests were considered significant when $p<0.05$, adopting a 95% confidence interval.

RESULTS

Anthropometric parameters for assessment of nutritional status

The mean values and standard deviations of the age and anthropometric parameters used to assess the nutritional status of the participants are presented in Table 1.

A statistically significant difference was observed in regard to weight and body mass index ($p<0.05$).

Table 1 - Mean values and standard deviations of age, body weight, height and BMI of the control group and women with obesity.

	Control (n=79)		Case (n=62)		
	Mean	SD	Mean	SD	p
Age (anos)	35.32	7.86	33.68	8.29	0,237
Body weight (kg)	56.95	5.52	106.07	16.46*	<0,001
Stature (m)	1.58	0.06	1.60	0.06	0,0150
BMI (kg/m^2)	22.37	1.60	41.59	6.05*	<0,001

Legend: * Significantly different values between obese patients and the control group, Student's t test or Mann-Whitney U-test ($p<0.05$). BMI = body mass index.

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Food consumption

The results obtained from the evaluation of food intake in relation to the amount of energy, macronutrients, and copper in the diets consumed by the study participants are presented in Table 2. It was possible to verify that there was no significant statistical difference between the groups (p>0.05) and that the intake of macronutrients was adequate according to the recommendations of the Institute of Medicine (2005). Additionally, the amount of copper in the diet was higher than the values established by the Institute of Medicine (2001) for both groups.

Table 2 - Mean values and standard deviations of energy, macronutrients and copper intake of obese women and control group.

Energy/Nutrients	Control (n=67) Mean ± DP	Case (n=30) Mean ± DP	p
Energy (Kcal)	1620, 16 ± 370,08	1617,51 ± 366,51	0,963
Carbohydrate (%)	51,13 ± 6,40	51,15 ± 7,35	0,720
Protein (%)	19,86 ± 3,28	20,22 ± 4,46	0,658
Lipid (%)	29,01 ± 5,05	28,62 ± 4,29	0,721
Copper (mg/day)	0,85 (0,29 – 2,06)	1,22 (0,37 – 9,84)*	<0,001

Legend: Student's t test or Mann-Whitney test (p<0.05). Reference values: 10 to 35% protein, 20 to 35% lipid, and 45 to 65% carbohydrates.(125) Reference values for copper: EAR = 0.7 mg Copper/day, age group 19 to 50 years (44).

Biochemical parameters for copper evaluation

Table 3 presents the mean values and standard deviations of the plasma and erythrocyte copper concentrations. The results revealed higher amounts of this mineral in the plasma of obese women and lower amounts in the erythrocytes compared to levels in the control group.

Table 3 - Mean values and standard deviations of the biochemical parameters of copper of the study participants.

Parameters	Control (n=79) Mean ± DP	Case (n=62) Mean ± DP	Value of p
Plasm copper (µg/dL)	93,98 ± 4,34*	112,67 ± 5,19	< 0,001
Erythrocyte copper (µg/dL)	73,73 ± 4,09*	57,76 ± 5,47	< 0,001

Legend: *Significantly different values between control and obese groups, Mann-Whitney test (p<0.05). Reference values for plasma copper are 80 to 155 µg/dL and for erythrocyte copper are 43 to 91 µg/dL. SD = standard deviation.

Table 4 presents the distribution of plasma and erythrocyte copper concentrations according to the BMI range. The results revealed that regardless of the degree of obesity of the women in the case group, they exhibited higher amounts of this mineral in the plasma and lower levels in the erythrocyte when compared to levels in the control group, and there was no statistical difference between the groups with grade II and grade III obesity.

Table 4 - Mean values and standard deviations of biochemical parameters of copper in the study participants according to BMI classification.

Parameters	Control (n=79) Mean ± DP	Grade II obesity (n=34) Mean ± DP	Grade III obesity (n=28) Mean ± DP	p
Plasm copper (µg/dL)	93,98 ± 4,34[a]	113,13 ± 5,16[b]	112,10 ± 5,26[b]	< 0,001
Erythrocyte copper (µg/dL)	73,73 ± 4,09[a]	57,56 ± 4,57[b]	58,01 ± 6,47[b]	< 0,001

Legend: ANOVA and post-hoc Bonferroni test and Turkey test (p<0.05). *Kruskal-wallis test (<0.05) Reference values for plasma copper are 80 to 155 µg/dL and for erythrocyte copper are 43 to 91 µg/dL. SD = standard deviation.

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Assessment of oxidative stress markers

Figure 1 presents the mean values and standard deviations of TBARS concentrations in the evaluated women. Women with obesity exhibited elevated TBARS values compared to those in the control group (p<0.001).

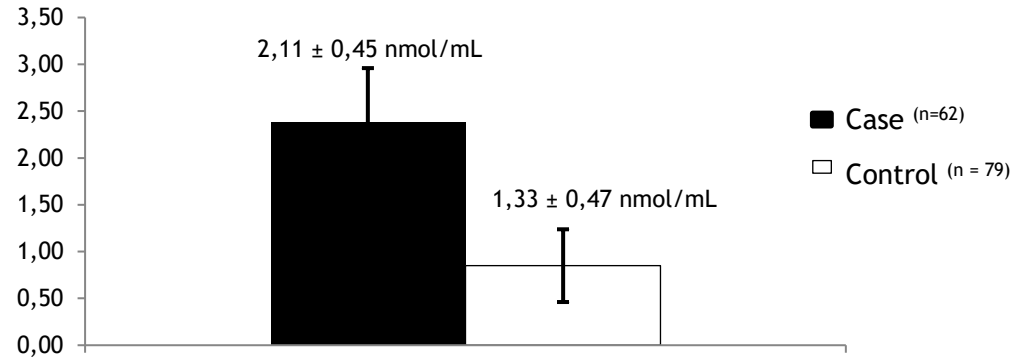

Figure 1 - Mean values and standard deviations of TBARS concentrations of obese women and control group. Test t de Student (p<0,001).

Table 5 presents the mean values and standard deviations of the enzyme activities in the antioxidant defense system. The results indicated reduced erythrocyte superoxide dismutase enzyme activity in obese women with a statistically significant difference between the groups.

Table 5 - Mean values and standard deviations of the activity of enzymes of the antioxidant defense system of the study participants.

Parameters	Control (n=79) Mean ± DP	Case (n=62) Mean ± DP	p
SOD (U/gHb)	2968,68 ± 576,20*	2396,87 ± 526,45	< 0,001
GPx (U/gHb)	42,50 ± 9,89	43,36 ± 11,94	0,774
CAT (mmol.min^{-1}.g^{-1}/g Hb)	2,53 ± 1,47	2,40 ± 1,31	0,572

Legend: *Significantly different values between control and obese groups, Student's t test or Mann-Whitney test (p<0.05). TBARS = thiobarbituric acid reactive substances; GPx = glutathione peroxidase; SOD = superoxide dismutase; CAT: catalase; SD = standard deviation.

Table 6 presents the distribution of oxidative stress markers of the participants according to BMI range. The results revealed that women with obesity grades II and III possessed elevated concentrations of TBARS in the plasma and also exhibited reduced activity of the enzyme superoxide dismutase in erythrocytes in comparison to these values in the control group; however, there was no statistical difference between the groups with obesity in relation to these markers of oxidative stress.

Additionally, there was no statistically significant difference in the activity of the enzymes glutathione peroxidase and catalase among the control, obesity grade II, and obesity grade III groups.

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Table 6 - Distribution of participants' oxidative stress markers copper in the study participants according to BMI classification.

Parameters	Control (n=79) Mean ± DP	Grade II obesity (n=34) Mean ± DP	Grade III obesity (n=28) Mean ± DP	p
TBARS (nmol/mL)*	1,33 ± 0,47[a]	2,02 ± 0,40[b]	2,22 ± 0,48[b]	< 0,001
SOD (U/gHb)	2968,68 ± 576,20[a]	2398,28 ± 511,96[b]	2395,17 ± 552,99[b]	< 0,001
GPx (U/gHb)*	42,50 ± 9,89[a]	43,08 ± 12,21[a]	43,69 ± 11,82[a]	0,779
CAT (mmol.min^{-1}.g^{-1}/g Hb)	2,54 ± 1,47[a]	2,45 ± 1,40[a]	2,34 ± 1,20[a]	1,000

Legend: ANOVA and post-hoc Bonferroni test and Turkey test (p<0.05). *Kruskal-wallis test (<0.05) TBARS = thiobarbituric acid reactive substances; GPx = glutathione peroxidase; SOD = superoxide dismutase; CAT: catalase; SD = standard deviation.

Relationship between nutritional status assessment parameters related to copper and oxidative stress markers

Table 7 presents the linear correlation analysis between the parameters of nutritional status evaluation related to copper and markers of oxidative stress in the case and control groups. It can be observed that in both groups there was a negative correlation between erythrocyte copper concentrations and erythrocyte superoxide dismutase antioxidant enzyme activity (p < 0.05).

Table 7 - Simple linear correlation analysis between copper concentrations and oxidative stress markers in the case and control groups.

Parameters	Control (n=79)			Case (n=62)		
	Dietary copper	Plasm copper	Erythrocyte copper	Dietary copper	Plasm copper	Erythrocyte copper
SOD (U/gHb)	0,089	-0,040	-0,244*	-0,138	0,116	-0,366*
GPX (U/gHb)	-0,195	0,013	-0,019	-0,150	0,008	-0,223
CAT (mmol.min^{-1}.g^{-1}/g Hb)	-0,028	-0,032	-0,076	-0,115	0,132	-0,238
TBARS (nmol/mL)	0,122	-0,003	-0,050	0,110	-0,020	-0,199

Legend: * Pearson's or Spearman's linear correlation coefficient (p<0.05). SOD = Superoxide dismutase; GPX = Glutathione Peroxidase; CAT = Catalase; TBARS = Thiobarbituric Acid Reactive Substances.

DISCUSSÃO

In this study, the dietary intake of energy, macronutrients, and copper in obese and eutrophic women was estimated, and the existence of a possible relationship between the parameters of oxidative stress and serum copper concentrations was investigated.

The results revealed that the amount of copper in the diet was higher than the values established by the Institute of Medicine for both groups. This result can be explained by the fact that obese women likely engage in a high consumption of foods considered sources of this mineral such as liver, chocolate, chocolate products, beans, peanuts, chestnuts, and potatoes (IBGE, 2011; Freire, Fisberg e Cozzolino, 2013; Forouzesh e colaboradores, 2021).

Similarly, in studies performed by Jiang e colaboradores (2020) and Lin e colaboradores (2008), was observed high copper content in the diets consumed by obese women with a mean values of 2.02 mg/day.

Although the obese womans had showed higher consumption of copper, the literature highlights that a ingestion between 0.8 and 2.4 mg per day this mineral constitutes a safe consumption, since do not result in an imbalance homeostasis of this in the body (Turnlund e colaboradores, 2005; Harvey e colaboradores, 2003).

With regard to copper plasma concentration analysis, it was observed that obese women had increased concentrations when compared to the control group, with a statistically significant difference (p<0,05).

These results are consistent with those found in the studies of Thillan e colaboradores (2021), Gu e colaboradores (2019) and Ge, Liu, and Liu (2020).

This researches also sugest a positive association between the serum copper concentrations and the BMI, however, the findings of the present study did not reveal a statistical difference between the plasma copper concentrations of the obese groups (grades II and III obesity).

High plasma copper concentration of obese individuals can be justified by probable increase of the serum concentrations of the cuproenzymes SSAO and ceruloplasmin in obesity that contributes to increased concentrations of this mineral in the plasma, since 95% this mineral is bound to this protein in the plasma (Kim e colaboradores, 2011; Yang e colaboradores, 2018).

In the sense, it should be noted that the low-grade chronic inflammation in obesity contribuites para in increased the ceruloplasmin concentrations in the plasm (Gu e colaboradores, 2019; Milanino e colaboradores, 1985).

Another factor that may explain the high copper concentrations in the plasma of obese women is the possible reduction in zinc bioavailability that has been evidenced in other studies, that describle a mechanism of competition for substrates between copper and zinc during the transport of such elements in the bloodstream, being that in situations of low zinc concentrations, there is an increase in serum copper concentrations. Moreover, reduced zinc concentrations in the plasm stimulates the production of inflammatory cytokines that increase the ceruloplasmin concentrations, and consequently the higher the amount of copper in this blood compartment (Gu e colaboradores, 2019; Prasad, 2014; Darroudi e colaboradores, 2019).

In relation to erythrocyte copper, reduced mineral concentrations were observed in obese women, with a statistically significant difference when compared to the control group, there was no difference observed in this biochemical parameter between the group obese women grade II and III. Regarding this result, it is important to mention the lack of data about this variable in the literature, and this limits further discussion. However, the reduction of copper in this blood component may be due to a process of retencion of this mineral in specific tissues such as the liver and adipose tissue (Yang e colaboradores, 2019; Vitoux, Arnoud e Chappuis, 1999).

The increase in copper concentrations in the liver tissue appears to be a contributing factor in regard to reducing the binding of this mineral to CCS, protein responsible for providing copper for the synthesis of the Cu-Zn SOD enzyme, and this consequently reduces the concentrations of this mineral in erythrocytes, since 60% of the copper content present in these cells is strongly linked to this antioxidant enzyme (Gu e colaboradores, 2019; Hatano, Nishi e Usui, 1982; Bertinato, Sherrard e Plouffe, 2010; Toro-Román e colaboradores, 2021).

In this study, it was also verified that women with obesity showed lower activity of the erythrocyte superoxide dismutase enzyme than did the control group (p<0.001) (Table 5), there was no diference between the women with grade II and III obesity (Table 6). Similarly, studies by Torkanlou e colaboradores (2016) and Nunes e colaboradores (2016) reported impairment of antioxidant defenses, and in particular, they observed a reduction in the activity of this enzyme in obese individuals. It should be noted that the erythrocyte superoxide dismutase is also considered to be a marker of nutritional status as related to copper, and this reinforces the change in distribution of this mineral in obese individuals.

The adipose tissue dysfunction impair the antioxidant defense system, since reduces the vailability of substrates necessary for the synthesis of enzymes that compose this system (Brown e colaboradores, 2009).

The increase in reactive oxygen species (ROS) generation and the low-grade chronic inflammation are also contributing factors to the degradation of the transcription factor NF-E2-related factor 2 (Nrf2) that plays an essential role in the synthesis of antioxidant enzymes (Chen e colaboradores, 2021).

The analysis of oxidative stress markers also revealed that obese women had higher TBARS concentrations than did the control group (p<0.001) (Figure 1), there was no difference between the group with grade II and III obesity (Table 7).

These results are consistent with those found in the studies of Horn e colaboradores

(2017) and Caimi e colaboradores (2019) which also showed increase in the concentration of this marker in obese individuals.

Based on these results, several factors may have contributed to the oxidative stress observed in obese individuals, including the adiposity, particularly the excess visceral fat, and the dysregulation on produced of synthesizing biologically active molecules such as plasminogen activator inhibitor-1 (PAI-1), TNF-α, leptin, and adiponectin in obesity (Furukawa e colaboradores, 2004).

In the analysis of linear correlation, was observed that the plasm and erythrocyte copper no appeared to influence the TBARS concentrations in obesity, since no was observed correlation between these parameters in both the groups. A likely justification for this result, refers to the fact that although there were changes in plasma and erythrocyte copper concentrations in women with obesity compared to the control group, these markers remained within the normal range established in the literature, exerting no significant influence on this marker of oxidative stress.

Another point to note concerning our results is that was observed negative correlation between erythrocytes copper concentrations and activity of the antioxidant enzyme erythrocyte superoxide dismutase in both groups ($p<0.05$) (Table 7).

In relation to this result, it is important to note that the literature does not report data similar to those observed in this study, since the association between erythrocyte copper concentration and the concentration and/or activity of the superoxide dismutase enzyme remains poorly investigated in studies involving individuals with obesity.

However, the literature provides some evidence that can contributes to the understanding of the mechanisms involved in the metabolic behavior of copper in this context. It has been suggested that the reduction in the activity of the enzyme superoxide dismutase associated with excess free radicals may induce the activation of genes involved in the synthesis of antioxidant enzymes as compensatory mechanism to strengthen the combat against oxidative stress (Chen e colaboradores, 2021).

In this process, minerals that make up the structure of these enzymes, such as copper, are recruited.

However, the greater movement of this mineral to the erythrocytes does not imply an increase in the synthesis of superoxide dismutase, since according to experimental studies the protein responsible for mediating this binding is inhibited due to the overload of copper in the hepatocytes (Bertinato, Sherrard e Plouffe, 2010).

CONCLUSION

The obese women evaluated in this study had a high intake of copper and also had altered nutritional status in relation to copper and oxidative stress.

However, although obese women possess high concentrations of copper in plasma, in this study it was not possible to identify the pro-oxidant action of this mineral, since the data did not reveal a correlation between markers of nutritional status related to copper and oxidative stress parameters.

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