Trace Elements and Electrolytes, Vol. 38 – No. 4/2021 (182-187)

Original

ISSN 0946-2104

DOI 10.5414/TEX01677
e-pub: July 26, 2021

Increased oxidative stress and alterations in the levels of some trace elements and minerals in obese/overweight subjects

Mohammed A. Hami[1] and Yasser Y. Al-Tamer[2]

[1]Department of Chemistry, Faculty of Science, University of Zakho, Kurdistan Region, and [2]Al-Noor University College, Mosul, Iraq

Key words
obesity – trace elements – body mass index – oxidative stress

Abstract. Introduction: Reactive oxygen species are considered to be important intracellular signaling molecules. However, these molecules, together with obesity, are risk factors for cardiovascular and metabolic diseases. This research attempts to explore the links among oxidative stress, trace elements, and obesity. Materials and methods: 160 male individuals were involved in this study. Six oxidative stress parameters were measured via enzyme-linked immunosorbent assay kits; glutathione, catalase, 8-iso-prostaglandin, malondialdehyde, vitamin C, and vitamin E. Trace elements were determined via flame atomic absorption spectrometry. Results: Significant differences in the levels of (calcium ($p = 0.0097$), magnesium ($p = 0.0005$), copper ($p = 0.0015$), zinc ($p = 0.0187$), glutathione ($p = 0.0276$), catalase ($p = 0.0329$), 8-iso-prostaglandin ($p = 0.0330$), malondialdehyde ($p = 0.0127$), and vitamin C ($p = 0.0263$)) among healthy, overweight, and obese groups. Furthermore, significant positive correlations were found between calcium, magnesium, and iron with most oxidative stress parameters. Conclusion: Oxidative stress is increased in obesity, and elevation in the levels of some trace elements increases oxidative stress. In addition, calcium, magnesium, and zinc are significantly decreased in populations with obesity, whereas serum copper was significantly increased in the obese group.

Accepted for publication
March 2, 2021

Correspondence to
Mohammed A. Hami, PhD
Department of Chemistry, Faculty of Science, University of Zakho, Kurdistan Region, Iraq
Mohammed.hami@uoz.edu.krd

Introduction

Based on a report from the World Health Organization in 2009, overweight and obesity come fifth in the list of leading risk factors that cause death in the world [1]. Obesity is a risk factor for cardiovascular diseases, diabetes, and cancer, altogether, killing ~ 1.2 million people in the United States alone in 2010 [2].

Obesity is a chronic metabolic disease that is caused by excessive food intake and less energy expenditure leading to accumulation of fat in adipose tissue and causing enlargement in size and number of adipose tissue cells [3, 4].

The term oxidative stress was first used in the journal Rubber Chemistry and Technology in 1956 [5]. It was in 1985 when oxidative stress was first defined as an imbalance between pro-oxidants, such as reactive oxygen species (ROS), and antioxidants, in favor of the former [6]. Due to their high reactivity, these ROS oxidize lipids, proteins, and DNA of the cells, leading to cell damage, apoptosis, and necrosis [7, 8]. It is known that oxidative stress is increased in obese individuals because of the increase in the level ROS, decreased antioxidant capacity, or the combination of both. The level of ROS is increased in obese individuals because of leukocyte infiltration, increased pro-inflammatory response [9], cytokine stimulation of cells, hypertrophic adipocytes, excess nutrient metabolism, and increased activation of Nicotinamide adenine dinucleotide phosphat (NADPH) oxidase. Decrease in antioxidant capacity occurs by chronic ingestion of fat-rich foods [10, 11].

Trace elements and macro-elements are molecules that perform crucial functions such as acting as catalyst for biochemical reactions and serving structural roles for hormones and enzymes [12, 13]. These elements act as a double-edged sword; on one hand, they reduce the oxidative stress by serving as co-factors for some antioxidant enzymes such as catalase, glutathione peroxidases, copper, zinc superoxide dismutase, and manganese superoxide dismutase [14].

For instance, magnesium acts as a co-factor for lipoprotein lipase, which plays an important role in chylomicrons metabolism [15, 16]. On the other hand, trace elements are involved in ROS production via many Fenton-type and Haber-Weiss reactions [17, 18], such as conversion of superoxide anion and hydrogen peroxide to extremely reactive hydroxyl radical with the help of iron [19].

As far as we are aware of, little attention has been paid on the interrelations among oxidative stress, trace elements, and BMI in male subjects. In this study, we attempt to illustrate the links among them.

Materials and methods

Study population

The study involved 160 male individuals who were aged 18 – 60 years (mean age = 36.8 ± 10.61 years) and with BMI ≥ 18.5. Oral and written consent were taken from the individuals participating in the study, and we adhered to the guidelines of the World Medical Association Declaration of Helsinki for studies involving human subjects [20].

The height and weight of all subjects were measured in order to calculate BMI. Height was measured with a portable stadiometer to the nearest 1 cm; weight was measured with a portable scale to the nearest 0.1 kg, and the individuals were lightly clothed and without shoes. The exclusion criteria were: taking medication or dietary supplements, alcohol, smoking, diabetes mellitus, thyroid diseases, hypertension, or any other disease or injury. The subjects were divided into three groups based on BMI: obese ≥ 30 BMI, overweight 25 – 29.9 BMI, and healthy subjects 18.5 – 24.9 BMI.

Analytical methods

After an overnight fast, ~ 8 mL venous blood samples were collected from the subjects. Then, the collected blood samples were centrifuged for ~ 15 minutes at 1,500 g, the separated sera were divided into 5 aliquots and were stored at –72 °C for nearly 1 month.

Total cholesterol (TC), triglyceride (TG), HDL cholesterol, albumin, total bilirubin, and uric acid were measured using COBAS 311 autoanalyzer (Roche Diagnostics, Mannheim, Germany). Albumin and total bilirubin were measured by colorimetric tests using the dye bromcresol green and diazonium ion, respectively. However, TC, TG, HDL cholesterol, and uric acid were measured by using enzymatic methods.

The elements iron, magnesium, zinc, copper, manganese, and calcium were measured using flame atomic absorption spectrometer (AAS, PerkinElmer PinAAcle 900T, Shelton, CT, USA) using deuterium background correction. Sample dilutions were performed with distilled water that had been deionized with a millipore water purification system. For determination of iron, copper, manganese, and zinc, the sera were diluted 5-fold with deionized water and 0.01% triton X-100 (Merck, Darmstadt, Germany). Whereas, for magnesium and calcium, the sera were diluted 30 times with deionized water and 0.01% triton X-100 and 0.05% lanthanum chloride.

Regarding oxidative stress parameter measurements, serum glutathione (GSH), serum catalase, serum 8-iso-prostaglandin (8-iso-PG), serum malondialdehyde (MDA), serum vitamin C, and serum vitamin E were determined by enzyme-linked immunosorbent assay kits (Sunlong Biotech Co., Ltd., Hangzhou, China) in accordance with the manufacturer's protocols.

Statistical analysis

GraphPad Prism software version 5.01 (GraphPad Software, Inc., La Jolla, CA, USA) were used for statistical analysis. All the parameter values are presented as mean ± standard deviation. Kolmogorov-Smirnov test was used to test normalization of data. One-way ANOVA and Kruskal-Wallis tests were used to compare three groups for normalized and non-normalized data, respectively. A p-value of < 0.05 was regarded as statistically significant [21]. The correlation between oxidative stress parameters with BMI and trace elements were determined by Pearson correlation coefficient and Spearman's correlation coefficient for normally and non-normally distributed data.

Results

The anthropometric and biochemical characteristics of the subjects are included in Tables

Table 1. Anthropometric characteristics of the subjects. Results are expressed as mean ± standard deviation.

	Control subjects (BMI < 25)	Overweight subjects (BMI 25 – 29.9)	Obese patients (BMI ≥ 30)	p-value
N	49	58	53	–
Age	32.91 ± 11.39	39.64 ± 9.39	40.85 ± 9.74	0.0002
BMI (kg/m^2)	22.47 ± 1.75	27.61 ± 1.42	33.65 ± 3.40	< 0.0001
Waist circumference (cm)	82.9 ± 9.6	94.1 ± 4.5	110.9 ± 5.8	< 0.0001

Table 2. Biochemical characteristics of the subjects. Results are expressed as mean ± standard deviation. Blood was drawn after 8 hours of fasting.

	Control subjects (BMI < 25)	Overweight subjects (BMI 25 – 29.9)	Obese subjects (BMI ≥ 30)	p-value
Cholesterol (mg/dL)	165.3 ± 35.44	173.6 ± 33.62	181.6 ± 37.45	0.0727
Triglyceride (mg/dL)	119.7 ± 67.15	195.3 ± 158.2	216.9 ± 118.0	**< 0.0001**
VLDL-cholesterol (mg/dL)	25.06 ± 14.98	39.09 ± 31.63	43.38 ± 23.57	**< 0.0001**
LDL-cholesterol (mg/dL)	102.4 ± 33.53	100.3 ± 34.82	106.9 ± 34.35	0.5838
HDL-cholesterol (mg/dL)	38.95 ± 8.63	34.28 ± 8.04	32.93 ± 6.59	**0.0004**
Uric acid (mg/dL)	5.12 ± 1.08	6.14 ± 1.05	6.17 ± 1.00	**< 0.0001**
Albumin (mg/dL)	4.82 ± 0.25	4.672 ± 0.24	4.63 ± 0.23	**0.0004**
Total bilirubin (mg/dL)	0.67 ± 0.5581	0.51 ± 0.2961	0.51 ± 0.28	0.2552
Calcium (mg/L)	100.1 ± 9.41	99.41 ± 11.60	94.61 ± 7.53	**0.0097**
Magnesium (mg/L)	8.22 ± 4.61	10.18 ± 3.88	6.84 ± 3.76	**0.0005**
Copper (mg/L)	0.82 ± 0.20	0.79 ± 0.22	0.94 ± 0.23	**0.0015**
Iron (mg/L)	0.48 ± 0.3	0.44 ± 0.15	0.50 ± 0.42	0.9
Zinc (µg/L)	0.68 ± 0.32	0.64 ± 0.38	0.32 ± 0.2	**0.0187**
Mn (µg/L)	0.66 ± 0.58	0.60 ± 0.36	0.70 ± 0.54	0.0554
Glutathione (µg/mL)	6.98 ± 3.02	2.42 ± 1.79	2.53 ± 1.38	**0.0276**
Catalase (ng/mL)	1.43 ± 1.44	0.69 ± 0.20	0.69 ± 0.57	**0.0329**
8-iso-prostaglandin (pg/mL)	14.84 ± 5.23	24.68 ± 10.52	30.47 ± 12.64	**0.0330**
Malondialdehyde (ng/mL)	58.16 ± 40.79	81.20 ± 18.2	105.3 ± 39.2	**0.0127**
Vitamin C (ng/mL)	13.52 ± 7.82	6.47 ± 5.11	8.72 ± 5.51	**0.0263**
Vitamin E (pg/mL)	7.24 ± 5.96	7.17 ± 8.82	6.68 ± 2.5	0.4811

Bold = significance different.

1 and 2. The Friedewald equation ([LDL-cholesterol] = [total cholesterol] – [HDL-cholesterol] – ([TG]/5)) was used to calculate LDL-cholesterol. The values of serum triglycerides were divided by 5 to calculate serum very low density lipoproteins (VLDL) cholesterol.

There were significant differences among the three groups in terms of BMI and waist circumference (Table 1). In addition, significant differences were demonstrated among the three groups for the following: serum triglyceride, VLDL cholesterol, HDL cholesterol, uric acid, albumin, magnesium, copper, zinc, glutathione, catalase, 8-iso-prostaglandin, malondialdehyde, and vitamin C (Table 2).

Regarding the correlation, there were significant positive correlations between the following: calcium-glutathione, calcium-8-iso-prostaglandin, calcium-malondialdehyde, magnesium-glutathione, magnesium-malondialdehyde, Mg-vitamin E, iron-glutathione, iron-8-iso-prostaglandin, iron-malondialdehyde, and manganese-8-iso-prostaglandin. However, significant negative correlations are observed between the followings; iron-vitamin C and manganese-catalase (Table 3).

Discussion

There are roughly 1.9 billion overweight people and 600 million people with obesity on the globe, therefore, thorough understanding the pathology of this disease is highly required [3]. In this research, we try to explore the links between BMI, trace elements, and oxidative stress parameters.

Table 3. Correlation analysis (Pearson or Spearman tests) between oxidative stress parameters with BMI and trace elements.

Correlations	Glutathione	Catalase	8-iso-prosta-glandin	Malondialdehyde	Vitamin C	Vitamin E
BMI	r = 0.10 p = 0.19	r = 0.068 p = 0.38	r = 0.040 p = 0.61	r = 0.022 p = 0.77	r = 0.001 p = 0.98	r = –0.013 p = 0.9071
Ca	**r = 0.17** **p = 0.028**	r = 0.098 p = 0.2076	**r = 0.19** **p = 0.011**	**r = 0.17** **p = 0.029**	r = 0.044 p = 0.57	r = 0.1812 p = 0.1011
Mg	**r = 0.20** **p = 0.017**	r = –0.05 p = 0.55	**r = 0.19** **p = 0.023**	**r = 0.21** **p = 0.0095**	r = –0.10 p = 0.23	**r = 0.2437** **p = 0.0264**
Cu	r = 0.085 p = 0.27	r = 0.033 p = 0.67	r = –0.067 p = 0.38	r = –0.063 p = 0.42	r = 0.11 p = 0.13	r = 0.0541 p = 0.6271
Fe	**r = 0.17** **p = 0.024**	r = –0.061 p = 0.43	**r = 0.17** **p = 0.03**	**r = 0.19** **p = 0.016**	**r = –0.17** **p = 0.028**	r = 0.0311 p = 0.7840
Zn	r = 0.049 p = 0.53	r = –0.086 p = 0.28	r = 0.056 p = 0.47	r = 0.064 p = 0.41	r = –0.057 p = 0.47	r = –0.026 p = 0.8154
Mn	r = –0.078 p = 0.33	**r = –0.21** **p = 0.0086**	r = 0.15 p = 0.054	r = –0.04 p = 0.62	r = –0.12 p = 0.12	r = 0.1048 p = 0.3645

r = correlation co-efficient. Bold = significance different.

In this study, there were significant differences in most trace elements (zinc, magnesium, calcium, and copper) level among the three groups. Low level of zinc could be due to urinary loss or poor-quality diet. The zinc element plays a key role in synthesis, storage, release, and activity of insulin. Thus, deficiency of zinc may cause insulin resistance causing development of obesity [22]. Furthermore, zinc binds to the molecule zinc-α2-glycoprotein, which plays an important role in lipid mobilization and lipolysis. Accordingly, deficiency of zinc would lead to decrease in lipolysis and accumulation of fat [23]. Magnesium is a co-factor for several enzymes that catalyze carbohydrate and lipid metabolism, such as phosphotransferases, lipoprotein lipase, and hydrolases; hence, depletion of these elements may cause accumulation of triglyceride [15, 24]. Our result of zinc and magnesium is in agreement with Suliburska et al. [22] and Fan et al. [25].

Copper level was significantly higher in individuals with obesity than in the control group. Our result is comparable with the study of Lima et al. [26, 27]. This could be due to excessive intake of food that contains copper, and also to the known antagonistic effect between zinc and copper [26].

The level of calcium was significantly lower in groups with obesity and overweight than in the control group, and this result agrees with the results of Jiao et al. [28]. This shows that calcium plays a main role in lipid metabolism. In a study by Vaskonen et al. [29], they showed that increased intake of calcium decreases the level of serum cholesterol, serum triglyceride, and LDL cholesterol. They suggested that calcium binds to fatty acids in the intestine preventing their absorption [29, 30].

The levels of vitamin C, catalase, and glutathione were significantly lower in the group with obesity than in the control group. The low level of glutathione could be due to its excessive oxidation by aldehyde compounds such as malondialdehyde [3]. The decrease in the level of vitamin C might be due to two factors, first enhanced consumption by ROS and secondly due to decrease in the level of glutathione since glutathione plays a major role in recycling of vitamin C. Our result is comparable to that of Garcia et al. [31].

Direct measurement of oxidative stress is not attainable because ROS are extremely reactive molecules and have very short half-lives. The only possible way is to measure the damage that these molecules make to the cell components [32]. The end products of this damage are molecules that can be measured easily such as malondialdehyde and 8-iso-prostaglandin [33, 34]. There were significant increases in the level of malondialdehyde and 8-iso-prostaglandin in overweight and obese subjects (Table 2). These results are comparable to those of Habib et al. [35] and Adnan et al. [36]. Increase in the level of these two biomarkers and decrease in the level of vitamin C, catalase, and gluta-

thione show that there is a cell injury due to attack by the ROS, i.e., oxidative stress [36].

There are strong correlations between some trace elements (calcium, magnesium, iron, and manganese) and oxidative stress (Table 3). Trace elements' involvement in oxidative stress could be via either involvements in Fenton reaction and Haber-Weiss reaction that produce ROS [37], or being a co-factor for antioxidant enzymes, or both [38]. Both ways lead to imbalance in pro-oxidant and antioxidant levels. Iron, for example, plays both roles in being a co-factor for some enzymes, such as catalase, and contributes in Fenton reaction [39, 40, 41]. Manganese and magnesium are co-factors for enzymatic antioxidant such as superoxide dismutase and catalase [16, 37, 39, 42]. In addition, calcium is also positively linked to oxidative stress. The mechanism behind this is that the aldosterone, which enhances reabsorption calcium, makes activation to xanthine oxidase and NADPH oxidase, leading to generation of ROS [43]. It is clear from the data of Table 3 that elements that correlate with pro-oxidant parameters are also correlated with some antioxidants. This shows that mobilization and activation of these antioxidants occur to compensate for the elevated levels of pro-oxidants.

Our result could have been more meaningful if we had analyzed the dietary intake (energy, mineral, and vitamins). Furthermore, measurement of superoxide dismutase and making correlations with the abovementioned trace elements could have also made our results more valuable and meaningful. Obviously, a larger number of subjects could have made the results more accurate.

Conclusion

In conclusion, there are strong relations between obesity and oxidative stress, zinc, magnesium, calcium, and copper. In addition, some trace elements, such as calcium, magnesium, iron, and manganese, contribute to the oxidative stress.

Acknowledgment

We would like to thank Dr. lazgin Abdi Jamil for helping us in all aspects of the study.

Funding

This work has been funded by the University of Zakho.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] *World Health Organization*. Global health risks: mortality and burden of disease attributable to selected major risks: Geneva: World Health Organization; 2009.

[2] *Dunham-Snary KJ, Ballinger SW*. Mitochondrial genetics and obesity: evolutionary adaptation and contemporary disease susceptibility. Free Radic Biol Med. 2013; *65:* 1229-1237. CrossRef PubMed

[3] *Bharathi V, Rengarajan RL, Radhakrishnan R, Hashem A, Abd Allah EF, Alqarawi AA, Anand AV*. Effects of a medicinal plant Macrotyloma uniflorum (Lam.) Verdc.formulation (MUF) on obesity-associated oxidative stress-induced liver injury. Saudi J Biol Sci. 2018; *25:* 1115-1121. CrossRef PubMed

[4] *Milagro FI, Moreno-Aliaga MJ, Martínez JA*. Nutrients, obesity and gene expression. Amsterdam: Elsevier; Principles of Nutrigenetics and Nutrigenomics. 2020. p. 431-440.

[5] *Ore S*. Oxidative stress relaxation of natural rubber vulcanized with Di-tertiary-butyl peroxide. Rubber Chem Technol. 1956; *29:* 1043-1046. CrossRef

[6] *Sies H*. Hydroperoxides and thiol oxidants in the study of oxidative stress in intact cells and organs. Oxidative stress. 1985; *1:* 73-90.

[7] *Pinton P*. Oxidative stress in cardiovascular diseases and obesity: role of p66Shc and protein kinase C. Oxidative medicine and cellular longevity. 2013; 2013.

[8] *Ortiz GG, Moisés FPP, Mireles-Ramírez M, Flores-Alvarado LJ, González-Usigli H, Sanchez-Gonzalez VJ, Sanchez-Lopez AL, Sánchez-Romero L, Díaz-Barba EI, Santoscoy-Gutiérrez JF*. Oxidative stress: love and hate history in central nervous system. Advances in protein chemistry and structural biology. Amsterdam: Elsevier; 2017. p. 1-31.

[9] *Huang C-J, McAllister MJ, Slusher AL, Webb HE, Mock JT, Acevedo EO*. Obesity-related oxidative stress: the impact of physical activity and diet manipulation. Sports Med Open. 2015; *1:* 32. CrossRef PubMed

[10] *Peairs A, Abbey E*. Antioxidants and inflammation in obesity. Bioactive Food as Dietary Interventions for Diabetes. Amsterdam: Elsevier; 2013. p. 413-434.

[11] *Vincent HK, Innes KE, Vincent KR*. Oxidative stress and potential interventions to reduce oxidative stress in overweight and obesity. Diabetes Obes Metab. 2007; *9:* 813-839. CrossRef PubMed

[12] *Fenzl V, Flegar-Meštrić Z, Perkov S, Andrišić L, Tatzber F, Žarković N, Duić Ž*. Trace elements and oxidative stress in hypertensive disorders of pregnancy. Arch Gynecol Obstet. 2013; *287:* 19-24. CrossRef PubMed

[13] *Hami MA, Ibrahim AA, Ahmed MS*. The status of serum zinc, magnesium and calcium in type 2 diabetic patients and their correlation with renal

function. Science Journal of University of Zakho. 2016; *4:* 208-212. CrossRef

[14] *Méplan C*. Trace elements and ageing, a genomic perspective using selenium as an example. J Trace Elem Med Biol. 2011; *25 (Suppl 1):* S11-S16. CrossRef PubMed

[15] *López-López P, Rojas-Sobarzo L, Arredondo-Olguín M*. Benefits of Selenium, Magnesium, and Zinc in Obesity and Metabolic Syndrome. Obesity. Amsterdam: Elsevier; 2018. p. 197-211.

[16] *Barbagallo M, Dominguez LJ*. Magnesium, oxidative stress, and aging muscle. Aging. Amsterdam: Elsevier; 2014. p. 157-166.

[17] *Lee J-C, Son Y-O, Pratheeshkumar P, Shi X*. Oxidative stress and metal carcinogenesis. Free Radic Biol Med. 2012; *53:* 742-757. CrossRef PubMed

[18] *Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M*. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact. 2006; *160:* 1-40. CrossRef PubMed

[19] *Puntarulo S*. Iron, oxidative stress and human health. Mol Aspects Med. 2005; *26:* 299-312. CrossRef PubMed

[20] *General Assembly of the World Medical Association*. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. J Am Coll Dent. 2014; *81:* 14-18. PubMed

[21] *Forthofer RN, Lee ES, Hernandez M*. Biostatistics: a guide to design, analysis and discovery. Amsterdam: Elsevier; 2006.

[22] *Suliburska J, Cofta S, Gajewska E, Kalmus G, Sobieska M, Samborski W, Krejpcio Z, Drzymala-Czyz S, Bogdanski P*. The evaluation of selected serum mineral concentrations and their association with insulin resistance in obese adolescents. Eur Rev Med Pharmacol Sci. 2013; *17:* 2396-2400. PubMed

[23] *Coverdale JPC, Khazaipoul S, Arya S, Stewart AJ, Blindauer CA*. Crosstalk between zinc and free fatty acids in plasma. Biochim Biophys Acta Mol Cell Biol Lipids. 2019; *1864:* 532-542. CrossRef PubMed

[24] *Zaakouk AM, Hassan MA, Tolba OA*. Serum magnesium status among obese children and adolescents. Egyptian Pediatric Association Gazette. 2016; *64:* 32-37. CrossRef

[25] *Fan Y, Zhang C, Bu J*. Relationship between selected serum metallic elements and obesity in children and adolescent in the US. Nutrients. 2017; *9:* 104. CrossRef

[26] *Lima SC, Arrais RF, Sales CH, Almeida MG, de Sena KC, Oliveira VT, de Andrade AS, Pedrosa LF*. Assessment of copper and lipid profile in obese children and adolescents. Biol Trace Elem Res. 2006; *114:* 19-29. CrossRef PubMed

[27] *Sánchez C, López-Jurado M, Aranda P, Llopis J*. Plasma levels of copper, manganese and selenium in an adult population in southern Spain: influence of age, obesity and lifestyle factors. Sci Total Environ. 2010; *408:* 1014-1020. CrossRef PubMed

[28] *Jiao H-T, Liu P, Lu W-T, Qiao M, Ren X-F, Zhang Z*. Correlation study between simple obesity and serum concentrations of essential elements. Trace Elem Electrolytes. 2014; *31:* 31. CrossRef

[29] *Vaskonen T, Mervaala E, Sumuvuori V, Seppänen-Laakso T, Karppanen H*. Effects of calcium and plant sterols on serum lipids in obese Zucker rats on a low-fat diet. Br J Nutr. 2002; *87:* 239-245. CrossRef PubMed

[30] *Cunha KA, Magalhães EIS, Loureiro LMR, Sant'Ana LFR, Ribeiro AQ, Novaes JF*. Ingestão de cálcio, níveis séricos de vitamina D e obesidade infantil: existe associação? Rev Paul Pediatr. 2015; *33:* 222-229. CrossRef PubMed

[31] *García OP, Ronquillo D, Caamaño MC, Camacho M, Long KZ, Rosado JL*. Zinc, vitamin A, and vitamin C status are associated with leptin concentrations and obesity in Mexican women: results from a cross-sectional study. Nutr Metab (Lond). 2012; *9:* 59. CrossRef PubMed

[32] *Codoñer-Franch P, Valls-Bellés V, Arilla-Codoñer A, Alonso-Iglesias E*. Oxidant mechanisms in childhood obesity: the link between inflammation and oxidative stress. Transl Res. 2011; *158:* 369-384. CrossRef PubMed

[33] *Bellanti F, Romano AD, Lo Buglio A, Castriotta V, Guglielmi G, Greco A, Serviddio G, Vendemiale G*. Oxidative stress is increased in sarcopenia and associated with cardiovascular disease risk in sarcopenic obesity. Maturitas. 2018; *109:* 6-12. CrossRef PubMed

[34] *Djelić N, Radaković M, Borozan S, Dimirijević-Srećković V, Pajović N, Vejnović B, Borozan N, Bankoglu EE, Stopper H, Stanimirović Z*. Oxidative stress and DNA damage in peripheral blood mononuclear cells from normal, obese, prediabetic and diabetic persons exposed to adrenaline in vitro. Mutat Res. 2019; *843:* 81-89. CrossRef PubMed

[35] *Habib SA, Saad EA, Elsharkawy AA, Attia ZR*. Pro-inflammatory adipocytokines, oxidative stress, insulin, Zn and Cu: Interrelations with obesity in Egyptian non-diabetic obese children and adolescents. Adv Med Sci. 2015; *60:* 179-185. CrossRef PubMed

[36] *Adnan MT, Amin MN, Uddin MG, Hussain MS, Sarwar MS, Hossain MK, Uddin SMN, Islam MS*. Increased concentration of serum MDA, decreased antioxidants and altered trace elements and macro-minerals are linked to obesity among Bangladeshi population. Diabetes Metab Syndr. 2019; *13:* 933-938. CrossRef PubMed

[37] *Stohs SJ, Bagchi D*. Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med. 1995; *18:* 321-336. CrossRef PubMed

[38] *Klotz L-O, Kröncke K-D, Buchczyk DP, Sies H*. Role of copper, zinc, selenium and tellurium in the cellular defense against oxidative and nitrosative stress. J Nutr. 2003; *133 (Suppl 1):* 1448S-1451S. CrossRef PubMed

[39] *Ribeiro TP, Fernandes C, Melo KV, Ferreira SS, Lessa JA, Franco RW, Schenk G, Pereira MD, Horn A Jr*. Iron, copper, and manganese complexes with in vitro superoxide dismutase and/or catalase activities that keep Saccharomyces cerevisiae cells alive under severe oxidative stress. Free Radic Biol Med. 2015; *80:* 67-76. CrossRef PubMed

[40] *Meroño T, Dauteuille C, Tetzlaff W, Martín M, Botta E, Lhomme M, Saez MS, Sorroche P, Boero L, Arbelbide J, Chapman MJ, Kontush A, Brites F*. Oxidative stress, HDL functionality and effects of intravenous iron administration in women with iron deficiency anemia. Clin Nutr. 2017; *36:* 552-558. CrossRef PubMed

[41] *Nikonorov AA, Skalnaya MG, Tinkov AA, Skalny AV*. Mutual interaction between iron homeostasis and obesity pathogenesis. J Trace Elem Med Biol. 2015; *30:* 207-214. CrossRef PubMed

[42] *Morais JBS, Severo JS, Santos LR, de Sousa Melo SR, de Oliveira Santos R, de Oliveira ARS, Cruz KJC, do Nascimento Marreiro D*. Role of magnesium in oxidative stress in individuals with obesity. Biol Trace Elem Res. 2017; *176:* 20-26. CrossRef PubMed

[43] *Schiffrin EL, Touyz RM*. Calcium, magnesium, and oxidative stress in hyperaldosteronism. Circulation. 2005; *111: 830*-831. PubMed