JML | ORIGINAL ARTICLE

Estimation of elemental concentrations in the toenail of young Saudi females with obesity

Hessah **Al-Muzafar** [1], Mohammed **Al-Hariri** [2]*

Author Affiliations

1. Department of Chemistry, College of Science, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia
2. Department of Physiology, College of Medicine, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

***Corresponding Author:**
Mohammed Al-Hariri,
Department of Physiology, College of Medicine,
Imam Abdulrahman Bin Faisal University,
Dammam, Saudi Arabia.
E-mail: mtalhariri@iau.edu.sa

DOI
10.25122/jml-2022-0017

Dates
Received: 9 December 2021
Accepted: 28 January 2022

ABSTRACT

Elemental homeostasis is essential for maintaining normal metabolic processes. Elements in the toenails are now considered in the diagnosis or screening and used as biomarkers of several metabolic disorders. The incidence of obesity is more prevalent in females than males globally. At the same time, females appeared more susceptible to elemental alterations than males. This study aimed to evaluate the variation in the levels of several elements in toenails as possible biomarkers of health conditions associated with obesity in young Saudi females. A cross-sectional study was performed, between February–November 2019. The study enrolled 79 young females divided into two groups: participants with obesity (n=39) and non-obese (n=40). The toenail was analyzed to estimate Fe, I, K, Na, Cd, Cr, Mn, Ca, Mg, Cu, Co, and Se levels. The study showed a significant elevation in the levels of Fe, Ca, K, and Na in the toenail sample of female participants with obesity compared to the non-obese group. The levels of Mn, Cd, Co, Cu, and Cr, were significantly decreased in the toenail of participants with obesity. Moreover, other elements (*i.e.*, Mg, I, and Se) were not significantly lower in the female group with obesity. Our findings confirmed the alterations of several elements among Saudi females with obesity. The toenail elemental analysis may become a useful diagnostic technique in monitoring the nutritional status, predicting some metabolic disorders, and environmental exposure.

KEYWORDS: Obesity, females, toenail, Saudi, elements, elemental concentrations.

INTRODUCTION

Obesity is one of the greatest health challenges worldwide and is associated with the incidence of many disorders such as hyperlipidemia, diabetes, cardiovascular diseases, metabolic syndrome, malignancies, and respiratory illnesses [1]. Alterations in the elemental concentrations, *i.e.*, metals and minerals, have been linked with obesity [2]. In Saudi Arabia, the estimated prevalence of obesity among females (≥15) is 36% [3].

Obesity is one of the main risk factors for morbidity and mortality due to non-communicable diseases. It is a preventable and modifiable risk factor for dyslipidemia, metabolic syndrome, and insulin resistance that may lead to disturbances in glycemic control [4].

Elemental homeostasis is essential for maintaining normal metabolic processes. Elements in the toenails are now considered in the diagnosis or screening and used as biomarkers of several metabolic disorders and environmental exposure [5]. Exposure to high levels of certain elements can affect the cell membrane, altering many cellular structures and/or functions such as transporters, enzymes, deoxyribonucleic acid, and signaling systems. In addition, the lack of some elements can compromise the overall health [6]. The nail samples benefit from a "time-integrated measure of exposure and body intake" because of the low growth rate of nails [7]. In addition, toenails are relatively sheltered from external or introduced contaminants through medication, chemicals, and shampooing. Moreover, human nails contain keratin-rich proteins that correlate proportionally based on their serum concentrations with elemental levels [8].

In clinical studies, using nails as markers for element status as a non-invasive specimen may increase the participation rate [9]. Other advantages include collecting and storage, making a study cost-effective [10]. In the same field, the assessment of most elements, 24-hour recall blood test, and dietary frequency questionnaires, cannot appropriately capture the real element concentrations in the body because of their minimal concentration and the continuous body homeostatic control. Thus, using nails to determine the element levels is preferred over other types of analysis [9, 11].

Healthy females have nearly double the amount of subcutaneous fat and fat storage than healthy males [12]. Severe obesity is more prevalent in females than males globally, and obesity-related outcomes are different in females and males [13]. At the same time, females appeared more susceptible to elemental alterations than males [14]. However, results concerning elemental alterations in obesity are still contradictory [15].

There is no reported data available on the elemental concentration in the nail of females in Saudi Arabia. Therefore, we aimed to investigate the variation in elemental levels in the toenails as possible biomarkers of health conditions associated with obesity in young Saudi females.

MATERIAL AND METHODS

Participant characteristics

A cross-sectional study was performed between February and November 2019 using convenience sampling and enrolling undergraduate female students at Imam Abdulrahman Bin Faisal University, Saudi Arabia. The eligibility criteria included healthy female participants (who did not have any chronic disease) and non-smokers. The study excluded overweight female participants (body mass index [BMI]=25.0 to <30), pregnant, and with a history of taking a mineral in the last month. The participants were divided into females with obesity (OG) BMI>30 kg/m^2) and normal, non-obese group (NOG), (BMI=18.5–25 kg/m^2) [16].

Determination of element contents in toenail samples

The free edge of the nail from the two great toes was taken by a stainless-steel nail clipper (pretreated with ethyl alcohol) on the same day for each participant. Toenail specimens (5 mg) from each participant were collected in a polyethylene bag either from naturally non-polished or after removing the nail polish before cutting the nails.

All samples of toenails underwent the washing procedure according to the protocol by Blazewicz *et al.* and Ishak *et al.* [17, 18]. Each washing stage took 10 min, and after drying in an oven (30 min at 100°C), they were weighed. The weighing stage was performed after washing and drying to prevent possible sample loss. All samples were carefully stored at 25°C [15].

A total of 5 mg of toenail sample was washed in series with H_2O_2 (30%), acetone, HNO_3 (65%), Triton-X 100 (Merck, Darmstadt, Germany). Afterward, the sample digestion and dilution procedures were carried out following the method described by Ishak and his colleagues in 2015 [17]. HNO_3 (0.5 mL) was added and left overnight at room temperature. The toenail specimens were kept in a drying oven (60°C) for one hour. After cooling, 0.2 mL of H_2O_2 was added, and all toenail specimens were incubated again for one hour in a drying oven (60°C). The mixtures were diluted to 10 mL using deionized water [17].

The analyses of the study elements [Iron (Fe), Iodine (I), Potassium (K), Sodium (Na), Cadmium (Cd), Chromium (Cr), Manganese (Mn), Calcium (Ca), Magnesium (Mg), Copper (Cu), Cobalt (Co) and Selenium (Se)] were carried out in the laboratory of Saudi Food & Drug Authority (SFDA) with simultaneous Inductively Coupled Plasma Emission spectrometers (ICPE-9800, ©Shimadzu Corporation, Japan). The spectrometer was optimized daily with a 10 µg/L solution of the study elements in a blank solution (1% HNO_3). The limits of detection were calculated as the level corresponding to three times the standard deviation of ten replicate measurements of the blank solution. All solutions of ICP elements (Merck, Germany) were prepared daily by the dissolution of the reference materials in water and used for calibration. Standards, blanks, and samples were measured with 103Rh as the internal standard (10 µg/L, Merck, Germany) [15]. Procedures on quality control were performed daily before and after each set of analyses using the certified reference material (Inductively Coupled Plasma Spectrometer, ©Shimadzu).

Statistical analysis

Data were analyzed using IBM SPSS, version 24. The normality of continuous variables was assessed using the Shapiro-Wilk test. Mean analysis was performed using the Mann-Whitney U test. Normally distributed data, *i.e.*, anthropometric data and age, were presented as mean and standard error of mean (SEM), whereas data with non-parametric distribution were expressed as median and 25–75 percentile boundaries. Statistical significance was considered at $p<0.05$.

RESULTS

Participant characteristics

Descriptive data of the study participants are shown in Table 1. There were 79 participants enrolled in this study, with 39 included in the OG, and 40 in NOG. The study results revealed that the mean BMI of the study participants in OG was 35.4±4.6 kg/m^2, and for NOG, it was 22.8±1.6 kg/m^2. The mean age of participants in NOG and OG were 20.5±1.6 and 21.3±1.8 years, respectively.

Elemental analysis

The levels of Ca ($p<0.001$), Fe ($p<0.001$), K ($p<0.001$), and Na ($p<0.001$) in the toenail of participants with OG were significantly higher as compared to participants in NOG. In contrast, Cd, Co, Cu, Cr, and Mn levels were significantly lower in the OG than in NOB ($p<0.001$). Furthermore, the levels of the other studied elements (Mg, I, and Se) were lower in OG as compared to NOG; however, those differences were not significant (Figure 1 and Table 2).

DISCUSSION

We found high levels of Fe, Ca, Na, and K and low levels of Cr, Cd, Co, Cu, and Mn in the toenail of females with obesity in Saudi Arabia. Alteration in the levels of these elements might play a vital role in the appearance and/or progression of several diseases. For example, reduced or elevated elements (Se, Cu, and Fe) may indicate the presence of depression in young people [19, 20].

Table 1. Participant characteristics.

	OG	NOG
Participants (#)	39	40
Mean age±SEM	21.3±1.8	20.5±1.6
BMI±SEM	35.4±4.6	22.8±1.6

OG – obese group; NOG – non obese group; SEM – standard error of mean; BMI – body mass index.

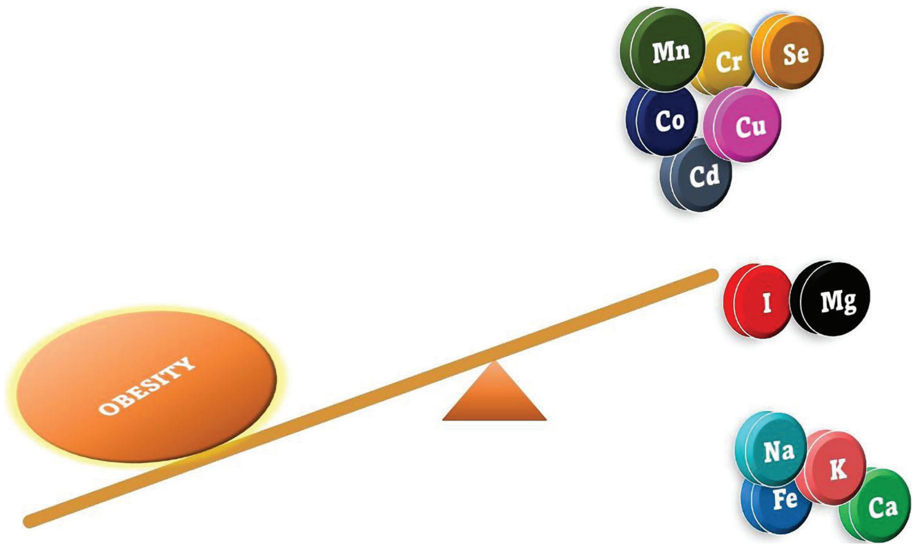

Figure 1. Elemental alteration in the toenails of young Saudi females with obesity.

The high level of serum ferritin is associated with the accumulation of adipose tissues independent of dietary iron intake [21]. Obesity-associated inflammatory signaling can cause a persistent elevation of blood hepcidin, resulting in decreased intestinal absorption of Fe and erythropoiesis as well as sequestration of Fe in metabolic tissues [22, 23], leading to subsequent accumulation of Fe in body tissue [24], including nails [25]. Therefore, these factors could explain our findings regarding the significant elevation of Fe in the toenails of females with obesity compared to the non-obese group.

In this study, the obese females showed a higher level of Ca than the non-obese females. Previous studies suggested some explanations for this metabolic disruption, including volumetric dilutional, physiological sequestration of vitamin D in adipose organs, adaptation to the need for more bone mass to support weight gain [26], and secondary hyperparathyroidism [27]. At the same point, obesity is believed to induce the production of inflammatory biomarkers that stimulate bone uptake by osteoclasts and may subsequently lead to higher serum Ca levels in obese individuals, which may, in turn, result in the mobilization of Ca from bone to other body compartments [28].

This study revealed a significant increase in the level of Na in the toenail of females with obesity. Earlier data reported an anomaly in the hormone mechanism associated with Na homeostasis that may contribute to the development of hypertension and hypernatremia in participants with obesity [29]. A previous review has explained the role of obesity-related hyperinsulinemia in Na retention. In this regard, insulin may increase the absorption of Na in the renal tubules and might increase Na accumulation [30].

In addition, the level of K in the toenail was significantly much higher among females with obesity than those in the NOG. K is an intracellular element necessary for the normal function of all living cells. Many researchers have investigated the associations between K and obesity. However, these observational results are considered controversial [30]. This may be explained partially by the contentious homeostatic regulation control of serum K. While blood and urine tend to show recent or current body status, the toenail sample may help assess a longer time frame of K level [30, 31]. However, the total body K level is directly associated with the body cell mass [32].

In this study, Cr, Cd, Co, and Mn were significantly lower in OG participants. These data are in accordance with earlier experimental reports [33, 34]. These findings encourage further studies concerning the use of biological sample as a biomarker of element status [35]. Obesity is associated with low-grade chronic inflammation, which can produce a variety of adipocytokines, [36] being positively correlated with element alteration [37]. Although elements in biological samples have been studied for many decades, the existing data are insufficient, and the effect of interactions on obesity is not often studied in practice [38].

It was reported that blood Cr was reduced in obese participants. Vice versa, Cr supplementation could be a promising agent in modulating obesity [38]. However, existing data on Cr status in obese participants are insufficient [39]. The existing data on the relationship between obesity and Cd are anecdotal [34]. Certain reports found a negative association between Cd and anthropometric indices of obesity [40]. Oppositely, others

Table 2. Comparison of studied elements (mg/kg) in the toenail samples of OG and NOG.

Element	OG Median (Interquartile range)	NOG Median (Interquartile range)	P-value
Ca	170.7 (120–240)	54.9 (20–180)	<0.001
Cd	1.5 (1.2–2.7)	3.5 (2.1–35.7)	<0.001
Co	7.5 (6.7–7.9)	10.1 (8.3–19.6)	<0.001
Cr	11.5 (10.7–12.0)	14.9 (8.3–19.6)	<0.001
Fe	40.3 (34.4–64.4)	35.9 (29.4–44.4)	<0.001
I	4.4 (2.9–7.3)	6.2 (4.4–13.5)	0.205
K	220.2 (180.8–260.4)	58.0 (23.2–250.2)	<0.001
Mg	28.1 (21.1–36.2)	30.8 (21.1–36.7)	0.963
Mn	0.3 (0.5–1.8)	0.7 (0.1–3.2)	<0.001
Na	131.0 (98.9–194.0)	39.2 (33.7–111.7)	<0.001
Se	8.3 (8.1–13.8)	16.5 (7.7–19.1)	0.190
Cu	12.4 (11.9–14.8)	17.6 (14.1–27.1)	<0.001

OG – obese group; NOG – non obese group.

failed to detect any significant relationship between the body-weight and Cd level [41].

Unfortunately, little is known about the effect of Co level on obesity in general. In the present study, the Co level in toenails from the OB group was lower than in NOB. The same finding was observed in another study using a plasma sample [38].

There is evidence of direct interaction between the elements. *i.e.*, "deficiency in one element may impair the absorption of another", [42] which could be the possible cause of the lower Cu toenail content observed in our study. The observation that lower Mn levels in the toenails of females with obesity was consistent with our previous study on Mn levels in a different biological sample [43].

The lower level of Se in the toenail of females with obesity was in agreement with a previous report that showed an inverse association between obesity and Se levels in nails [44]. However, the statistical analysis revealed no significant difference at the median level of Se in both groups. Perhaps this could be due to the relatively small size of the sample [45].

Moreover, despite the absence of statistically significant differences, the observed decrease in levels of Mg and I are generally consistent with findings from previous studies where it was reported that obesity increases the incidence of Mg [46] and I deficiency [47].

CONCLUSION

The present data added new information on the elements from the toenails of young Saudi females with obesity. The elemental analysis of the nail may become a useful diagnostic technique in monitoring nutritional status, predicting some metabolic disorders, and environmental exposure in the future. However, further studies are needed with a representative sample size to precisely elaborate reference ranges of elements in the nail and formulate universal guidance on methodology considering age, gender, and lifestyle. The medical care plan for obese individuals should include behavioral counseling to reduce their exposure to high-calorie foods with low nutritional value. To the best of our knowledge, this is the first study that reports elemental alterations in the toenail of females with obesity in Saudi Arabia. Expert lab technicians used a very advanced automated instrument for the elemental analysis in a qualified and specialized governmental institute (SFDA).

There were some limitations in our study. Firstly, the study did not correlate the results with demographic variables of other health and lifestyle data of the study sample, which are essential in future data collection. Secondly, there was a small sample size, and the focus was on only one gender and a specific age group. Third, we did not correlate the elemental levels in toenails with blood levels.

ACKNOWLEDGMENTS

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

The study proposal was approved by the local Review Board (IRB-2018-01-028-31/1/2018).

Consent to participate

A written informed consent was taken from all participants.

Authorship

MA and HA contributed to the conceptualization of the study. HA contributed to supervision, methodology, and investigation. MA contributed to validation, analysis, and writing of original draft. Both authors read and agreed to the published version of the manuscript.

REFERENCES

1. Albaker W, El-Ashker S, Baraka MA, El-Tanahi N, *et al.* Adiposity and Cardiometabolic Risk assessment Among University Students in Saudi Arabia. Sci Prog. 2021; 104:36850421998532. doi.org/10.1177/0036850421998532.
2. Al-Muzafar HM, Al-Hariri MT. Alterations in manganese level in the biological samples of young obese Saudi women. J Taibah Univ Med Sci. 2021; 16:706–11. doi.org/10.1016/j.jtumed.2021.04.013.
3. World Health Organization. Obesity and overweight. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
4. Alkahtani S, Elkilany A, Alhariri M. Association between sedentary and physical activity patterns and risk factors of metabolic syndrome in Saudi men: A cross-sectional study. BMC Public Health. 2015; 15:1234. doi.org/10.1186/s12889-015-2578-4.
5. Gutiérrez-González E, García-Esquinas E, de Larrea-Baz NF, Salcedo-Bellido I, *et al.* Toenails as biomarker of exposure to essential trace metals: A review. Environ Res. 2019; 179:108787. doi.org/10.1016/j.envres.2019.108787.
6. Fraga CG, Oteiza PI, Keen CL. Trace elements and human health. Mol Aspects Med. 2005; 26:233–234. doi.org/10.1016/j.mam.2005.07.014.
7. Mueller WH. Biological markers in epidemiology. Am J Hum Biol. 1991; 3:218–219. doi.org/10.1002/ajhb.1310030225.
8. Saeedi P, Shavandi A, Meredith-Jones K. Nail Properties and Bone Health: A Review. J Funct Biomater. 2018 Apr 23;9(2):31. doi: 10.3390/jfb9020031.
9. He K. Trace elements in nails as biomarkers in clinical research. Eur J Clin Invest. 2011 Jan;41(1):98-102. doi: 10.1111/j.1365-2362.2010.02373.x.
10. Bulska E, Ruszczyńska A. Analytical Techniques for Trace Element Determination. Physical Sciences Reviews. 2017; 2(5):20178002. doi.org/10.1515/psr-2017-8002.
11. Xun P, Liu K, Morris JS, Daviglus ML, He K. Longitudinal association between toenail selenium levels and measures of subclinical atherosclerosis: the CARDIA trace element study. Atherosclerosis. 2010; 210(2):662-7. doi.org/10.1016/j.atherosclerosis.2010.01.021.
12. Blaak E. Gender differences in fat metabolism. Curr Opin Clin Nutr Metab Care. 2001 Nov;4(6):499-502. doi: 10.1097/00075197-200111000-00006.
13. Leeners B, Geary N, Tobler PN, Asarian L. Ovarian hormones and obesity. Hum Reprod Update. 2017 May 1;23(3):300-321. doi: 10.1093/humupd/dmw045.
14. Berglund M, Lindberg AL, Rahman M, Yunus M, *et al.* Gender and age differences in mixed metal exposure and urinary excretion. Environ Res. 2011; 111:1271–1279. doi.org/10.1016/j.envres.2011.09.002.
15. Sureda A, Bibiloni MDM, Julibert A, Aparicio-Ugarriza R, *et al.* Trace element contents in toenails are related to regular physical activity in older adults. PLOS ONE. 2017;12(10):e0185318. doi: 10.1371/journal.pone.0185318.
16. El-Ashker S, Pednekar MS, Narake SS, Albaker W, Al-Hariri M. Blood Pressure and Cardio-Metabolic Risk Profile in Young Saudi Males in a University Setting. Medicina. 2021; 57:755. doi.org/10.3390/medicina57080755.
17. Błażewicz A, Liao K-Y, Liao H-H, Niziński P, *et al.* Alterations of Hair and Nail Content of Selected Trace Elements in Nonoccupationally Exposed Patients with Chronic Depression from Different Geographical Regions. Biomed Res Int. 2017; 2017:3178784. doi.org/10.1155/2017/3178784.
18. Ishak I, Rosli FD, Mohamed J, Mohd Ismail MF. Comparison of Digestion Methods for the Determination of Trace Elements and Heavy Metals in Human Hair and Nails. Malays J Med Sci. 2015; 22(6):11–20.
19. Fraga CG. Relevance, essentiality and toxicity of trace elements in human health. Mol Aspects Med. 2005; 26:235–244. doi.org/10.1016/j.mam.2005.07.013.
20. Donma M, Donma O. Trace elements and physical activity in children and adolescents with depression. Turk J Med Sci. 2010;40. doi: 10.3906/sag-0811-33.
21. Moore Heslin A, O'Donnell A, Buffini M, Nugent AP, *et al.* Risk of Iron Overload in Obesity and Implications in Metabolic Health. Nutrients. 2021 May 2;13(5):1539. doi: 10.3390/nu13051539.
22. Park CH, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem. 2001; 276:7806–7810. doi.org/10.1074/jbc.M008922200.
23. Jehn M, Clark JM, Guallar E. Serum ferritin and risk of the metabolic syndrome in US adults. Diabetes Care. 2004; 27:2422–2428. doi.org/10.2337/diacare.27.10.2422.
24. Al-Hakeim HK. Correlation between Iron Status Parameters and Hormone Levels in Women with Polycystic Ovary Syndrome. Clin Med Insights Womens Health. 2012;5:CMWH.S8780. doi: 10.4137/CMWH.S8780.
25. Chaturvedi R, Banerjee S, Chattopadhyay P, Bhattacharjee CR, *et al.* High iron accumulation in hair and nail of people living in iron affected

areas of Assam, India. Ecotoxicol Environ Saf. 2014; 110:216–220. doi.org/10.1016/j.ecoenv.2014.08.028.
26. Drincic AT, Armas LAG, Van Diest EE, Heaney RP. Volumetric dilution, rather than sequestration best explains the low vitamin D status of obesity. Obesity. 2012; 20:1444–1448. doi.org/10.1038/oby.2011.404.
27. Hamoui N, Anthone G, Crookes PF. Calcium metabolism in the morbidly obese. Obes Surg. 2004; 14:9–12. doi.org/10.1381/096089204772787211.
28. Timerga A, Haile K. Patterns of Calcium- and Chloride-Ion Disorders and Predictors among Obese Outpatient Adults in Southern Ethiopia. Diabetes Metab Syndr Obes. 2021; 14:1349–1358. doi.org/10.2147/DMSO.S300434.
29. Reisin E. Sodium and obesity in the pathogenesis of hypertension. Am J Hypertens. 1990; 3:164–167. doi.org/10.1093/ajh/3.2.164.
30. Cai X, Li X, Fan W, Yu W, *et al.* Potassium and Obesity/Metabolic Syndrome: A Systematic Review and Meta-Analysis of the Epidemiological Evidence. Nutrients. 2016; 8:183. doi.org/10.3390/nu8040183.
31. Zupo R, Castellana F, Boninfante B, Lampignano L, *et al.* Uric Acid and Potassium Serum Levels Are Independent Predictors of Blood Pressure Non-Dipping in Overweight or Obese Subjects. Nutrients. 2019 Dec 5;11(12):2970. doi: 10.3390/nu11122970.
32. Pierson RN, Lin DH, Phillips RA. Total-body potassium in health: effects of age, sex, height, and fat. Am J Physiol. 1974; 226(1):206–12. doi.org/10.1152/ajplegacy.1974.226.1.206.
33. Song M, Schuschke DA, Zhou Z, Chen T, *et al.* High fructose feeding induces copper deficiency in Sprague-Dawley rats: a novel mechanism for obesity related fatty liver. J Hepatol. 2012; 56:433–440. doi.org/10.1016/j.jhep.2011.05.030.
34. Tinkov AA, Filippini T, Ajsuvakova OP, Aaseth J, *et al.* The role of cadmium in obesity and diabetes. Sci Total Environ. 2017 Dec 1;601-602:741-755. doi: 10.1016/j.scitotenv.2017.05.224
35. Failla ML. Considerations for determining "optimal nutrition" for copper, zinc, manganese and molybdenum. Proc Nutr Soc. 1999; 58:497–505. doi.org/10.1017/s0029665199000646.
36. Castro AM, Macedo-de la Concha LE, Pantoja-Meléndez CA. Low-grade inflammation and its relation to obesity and chronic degenerative diseases. Revista Médica Del Hospital General de México. 2017; 80:101–105. doi.org/10.1016/j.hgmx.2016.06.011.
37. Palladini G, Ferrigno A, Di Pasqua LG, Berardo C, *et al.* Associations between serum trace elements and inflammation in two animal models of nonalcoholic fatty liver disease. PLoS One 2020;15:e0243179. https://doi.org/10.1371/journal.pone.0243179.
38. Błażewicz A, Klatka M, Astel A, Partyka M, Kocjan R. Differences in trace metal concentrations (Co, Cu, Fe, Mn, Zn, Cd, And Ni) in whole blood, plasma, and urine of obese and nonobese children. Biol Trace Elem Res. 2013; 155:190–200. doi.org/10.1007/s12011-013-9783-8.
39. Jamilian M, Foroozanfard F, Kavossian E, Kia M, *et al.* Effects of Chromium and Carnitine Co-supplementation on Body Weight and Metabolic Profiles in Overweight and Obese Women with Polycystic Ovary Syndrome: a Randomized, Double-Blind, Placebo-Controlled Trial. Biol Trace Elem Res. 2020; 193:334–341. doi.org/10.1007/s12011-019-01720-8.
40. Nie X, Wang N, Chen Y, Chen C, *et al.* Blood cadmium in Chinese adults and its relationships with diabetes and obesity. Environ Sci Pollut Res Int. 2016; 23:18714–18723. doi.org/10.1007/s11356-016-7078-2.
41. Tinkov AA, Gatiatulina ER, Popova EV, Polyakova VS, *et al.* Early High-Fat Feeding Induces Alteration of Trace Element Content in Tissues of Juvenile Male Wistar Rats. Biol Trace Elem Res. 2017; 175:367–374. doi.org/10.1007/s12011-016-0777-1.
42. Milner JA. Trace minerals in the nutrition of children. J Pediatr. 1990 Aug;117(2 Pt 2):S147-55. doi: 10.1016/s0022-3476(05)80013-7.
43. Al-Muzafar HM, Al-Hariri MT. Elements alteration in scalp hair of young obese Saudi females. Arab J Basic Appl Sci. 2021; 28:122–127. doi.org/10.1080/25765299.2021.1911070.
44. Xu R, Chen C, Zhou Y, Zhang X, Wan Y. Fingernail selenium levels in relation to the risk of obesity in Chinese children: A cross-sectional study. Medicine (Baltimore). 2018 Mar;97(9):e0027. doi: 10.1097/MD.0000000000010027.
45. Faber J, Fonseca LM. How sample size influences research outcomes. Dental Press J Orthod. 2014; 19:27–29. doi.org/10.1590/2176-9451.19.4.027-029.ebo.
46. Hassan SAU, Ahmed I, Nasrullah A, Haq S, *et al.* Comparison of Serum Magnesium Levels in Overweight and Obese Children and Normal Weight Children. Cureus. 2017 Aug 24;9(8):e1607. doi: 10.7759/cureus.1607.
47. Lecube A, Zafon C, Gromaz A, Fort JM, *et al.* Iodine deficiency is higher in morbid obesity in comparison with late after bariatric surgery and non-obese women. Obes Surg. 2015; 25(1):85-9. doi.org/10.1007/s11695-014-1313-z.