



Original article

Plasma concentration of IL-6 and TNF- α and its relationship with zincemia in obese women[☆]

Maura Cristina Porto Feitosa^a, Vanessa Batista de Sousa Lima^a,
José Machado Moita Neto^b, Dilina do Nascimento Marreiro^{a,*}

^a Department of Nutrition, Universidade Federal do Piauí, Teresina, PI, Brazil

^b Department of Chemistry, Universidade Federal do Piauí, Teresina, PI, Brazil

ARTICLE INFO

Article history:

Received 6 August 2012

Accepted 23 March 2013

Available online 14 September 2013

Keywords:

Obesity

Zinc

Inflammation

Interleukin-6

Tumor necrosis factor- α

ABSTRACT

Objective: In obesity, the excessive adipose tissue increases the synthesis of inflammatory cytokines, which appear to alter the metabolism of minerals, such as zinc. However, the mechanisms involved remain unclear. This study investigated whether the concentrations of interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) in plasma can influence biochemical parameters of zinc in obese women.

Methods: Seventy-six pre-menopausal women, aged between 20 and 50 years, were divided into two groups: the case group, composed of obese women ($n = 37$) and the control group, composed of non-obese women ($n = 39$). Analysis of the plasmatic and erythrocytary zinc, and plasmatic cytokines were conducted by flame atomic absorption spectrophotometry and by ELISA, respectively.

Results: The plasmatic zinc and concentrations of IL-6 in plasma did not show significant differences between obese women and controls ($p > 0.05$). The erythrocytary zinc was $36.4 \pm 15.0 \mu\text{g/gHb}$ in the case group, and $45.4 \pm 14.3 \mu\text{g/gHb}$ ($p = 0.025$) in the control group. The concentrations of TNF- α in plasma were $42.0 \pm 11.9 \text{ pg/mL}$ and $19.0 \pm 1.0 \text{ pg/mL}$ in obese women and in controls, respectively ($p < 0.001$). The plasmatic zinc had a significant negative correlation with the values of TNF- α ($r = -0.44$, $p = 0.015$).

Conclusion: Obese women presented lower concentrations of erythrocytary zinc than the control group. The study demonstrated a probable influence of the inflammatory process on metabolism of zinc in obese patients.

© 2013 Elsevier Editora Ltda. All rights reserved.

Concentrações plasmáticas de IL-6 e TNF- α e sua relação com a zincemia em mulheres obesas

RESUMO

Objetivo: Na obesidade, o excesso de tecido adiposo aumenta a síntese de citocinas inflamatórias que parecem alterar o metabolismo de minerais, como o zinco. Entretanto, os mecanismos envolvidos neste processo ainda não estão totalmente esclarecidos. O estudo

Palavras-chave:

Obesidade

Zinco

[☆] Study conducted at the Department of Nutrition, Universidade Federal do Piauí, Teresina, PI, Brazil.

* Corresponding author.

E-mail: dilina.marreiro@gmail.com (D.N. Marreiro).

0104-4230/\$ – see front matter © 2013 Elsevier Editora Ltda. All rights reserved.

<http://dx.doi.org/10.1016/j.ramb.2013.03.003>

Inflamação
Interleucina-6
Fator de necrose tumoral- α

investigou se as concentrações de interleucina-6 (IL-6) e fator de necrose tumoral- α (TNF- α) no plasma podem influenciar os parâmetros bioquímicos do zinco.

Métodos: Setenta e três mulheres, com idade entre 20 e 50 anos, foram distribuídas em dois grupos: grupo caso, composto de obesas ($n=37$) e grupo controle, composto de não obesas ($n=39$). Análises de zinco plasmático e eritrocitário e as citocinas inflamatórias plasmáticas foram realizadas pelo método da espectrofotometria de absorção atômica e pelo ELISA, respectivamente.

Resultados: O zinco plasmático e as concentrações de IL-6 no plasma não mostraram diferenças significativas entre as mulheres obesas e grupo controle ($p>0,05$). O valor do zinco eritrocitário foi $36,4 \pm 15,0 \mu\text{g/gHb}$ nas mulheres obesas e $45,4 \pm 14,3 \mu\text{g/gHb}$ ($p=0,025$) no grupo controle. As concentrações de TNF- α no plasma foram $42,0 \pm 11,9 \text{ pg/mL}$ e $19,0 \pm 1,0 \text{ pg/mL}$ nas mulheres obesas e controles, respectivamente ($p<0,001$). O zinco plasmático apresentou uma correlação negativa significativa com os valores de TNF- α ($r=-0,44$, $p=0,015$).

Conclusão: As mulheres obesas apresentaram menores concentrações de zinco eritrocitário em relação ao grupo controle. O estudo mostra uma provável influência do processo inflamatório no metabolismo do zinco em pacientes obesos.

© 2013 Elsevier Editora Ltda. Todos os direitos reservados.

Introduction

Obesity is directly related to changes in the endocrine and metabolic functions of adipose tissue. In obese individuals, this tissue increases the capacity for synthesis of adipocytokines or adipokines with proinflammatory action. Studies have identified that metabolic disturbances in obesity are related to macronutrient levels. Recently, there has been an increased interest on the contribution of minerals in various pathophysiological changes associated with obesity.¹⁻³

Zinc is one of the most important minerals for metabolism. Among its biological functions, this element is a cofactor of over 300 metalloenzymes; it acts in the catalytic activity of several enzymes, such as carbonic anhydrase, alcohol dehydrogenase, alkaline phosphatase, and enzymes involved in the metabolism of carbohydrates, lipids, and proteins.^{4,5}

Tissues of obese animals have low concentrations of micronutrients, such as zinc, copper, iron, and manganese. Kennedy and Failla⁶ observed low concentrations of zinc in the skin, muscle, pancreas, and bone of obese mice. Moreover, the liver, intestine, and adipose tissues had higher zinc concentrations, suggesting a possible change in the distribution of the mineral in the obese.

A relationship between zinc status and cytokine production is reported in disorders associated with chronic inflammation, such as obesity. This process may occur through an increase in the content of hepatic zinc at the expense of plasmatic zinc. Elevated concentrations of glucocorticoids lead to a reduction of plasmatic zinc and an increase in zinc uptake by the liver. These data show the involvement of synergistic participation of interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and glucocorticoids on the metabolic behavior of zinc; the combination of these elements depend on an adequate zinc content in the liver.^{7,8}

In humans, inflammatory cytokines have been demonstrated to both up- and down-regulate the expression of specific cellular zinc transporters (ZnT's and Zip's), in response to an increasing demand for zinc in inflammation.^{9,10} In these processes, there is an increase of expression of encoding

genes for zinc-transporter protein Zip-14, which promotes the uptake of zinc from the extracellular compartment to the interior of the cells. In obesity, a high concentration of IL-6 in plasma promotes an increase in gene expression of this transporter, which may alter the distribution of zinc in cellular compartments.^{7,11} Studies have demonstrated a rapid decrease in zinc plasma concentration that was preceded by prominent increases in concentration of TNF- α and IL-6 in plasma in humans exposed to inflammation.^{9,12,13}

Considering the importance of obesity as a chronic disease, the secretion of adipocytokines and other proteins in the adipose tissue, and the possible influence of these metabolites on the homeostasis of zinc, this study aimed to investigate the relationship between biochemical parameters of zinc and the concentrations of IL-6 and of TNF- α in the plasma of obese women.

Methods

This was a clinical and case-control study, involving 76 randomly selected adult females, aged between 20 and 50 years, who sought treatment at an endocrinology clinic. This sample derived from the study by Ferro et al.¹⁴ The analysis of concentrations of proinflammatory cytokines in plasma was performed from a biobank of samples arising from the aforementioned research,¹⁴ developed in the Universidade Federal do Piauí (UFPI) by researchers involved in this study.

The obese women ($n=37$) who presented at the clinic were selected for the study if they met the following criteria: their body mass index (BMI) was higher than 30 kg/m^2 ; they were not taking any vitamin-mineral supplementation and/or other medicines; they did not have any illnesses that could interfere with zinc-related nutritional status and metabolic profile, such as diabetes mellitus, chronic renal insufficiency, chronic diarrhea, and malabsorption syndrome; and were nonsmokers. The control group ($n=39$) was selected according to the same criteria as the obese women, but had a BMI between 18.5 kg/m^2 and 24.9 kg/m^2 . The study was approved by the

Ethics Committee of the UFPI, and the individuals signed an informed consent.

Assessment of nutritional status

BMI was calculated using measures of weight in kilograms and height in meters. The classification of obesity according to BMI was conducted in agreement with the criteria of the World Health Organization.¹⁵

Collection of biological material and biochemical parameters

Blood samples (25 mL) were taken in the morning, from 7:30 am to 9:00 am, after at least a 12-hour fast. The plasma was separated from the total blood by centrifugation at 1,831 xg for 15 minutes at 4 °C (SIGMA 2K15 centrifuge). Three aliquots of each plasma sample were diluted at a ratio of 1:4 with Milli-Q® water and aspirated directly into the flame of the atomic absorption spectrophotometer.¹⁶ Tryptizol® (Merck), prepared by dilution with Milli-Q® water with 3% glycerol at 0.1, 0.2, 0.3, 0.5, and 1.0 µg/mL dilutions were used as a standard.

For the separation of the erythrocytes, the erythrocytary mass obtained from total blood was washed three times with 5 mL of 0.9% saline solution, homogenized by inversion, and centrifuged at 2,493 xg for 10 minutes (Sorvall® RC-SB) at 4 °C. After the last centrifugation, the saline solution was aspirated, and the erythrocytary mass was carefully extracted using a micropipette, placed in demineralized Eppendorf tubes, and stored at -20 °C for zinc and hemoglobin analysis.¹⁷ To express the results in terms of mass zinc/mass of hemoglobin (µg/gHb), the erythrocytary lysis was measured according to the cyanmethemoglobin method.¹⁸

The erythrocyte analysis was performed using atomic absorption spectrophotometry.¹² Tryptizol® was used as a reference, prepared by dilution in Milli-Q® water at concentrations of 0.1, 0.2, 0.3, 0.5, and 1.0 µg/mL. The reference interval for plasmatic and erythrocytary zinc is 70-110 µg/dL and 40-44 µg/gHb, respectively.^{19,20}

The concentrations of IL-6 and of TNF-α in plasma were performed by enzyme linked immunosorbent assay (ELISA), using the Lincoplex Cytokine Analytes Kit (Linco Research - Missouri, USA), according to the methodology recommended by the manufacturer. The limits of detection for IL-6 and TNF-α were 0.3 to 0.7 pg/mL and 0.1 pg/mL, respectively.

Statistical analysis

Data were processed and analyzed using the S-PLUS software for Windows, version 3.2, and Minitab Release, version 11.0 for Windows 9.0. Student's t-test and Mann-Whitney tests were used to determine whether differences on the main study variables existed between groups. Pearson's correlation analysis was applied to establish the correlation of concentrations of IL-6 and TNF-α in plasma with the plasmatic and erythrocytary zinc. Significance was established at $p < 0.05$ a priori for all statistical tests.

Results

The subject's characteristics are shown in Table 1. In this study, a significant difference between the case and control groups was observed regarding erythrocytary zinc and the plasmatic TNF-α, as demonstrated in Table 2, and a significant negative correlation between plasmatic zinc and TNF-α in obese patients ($r = -0.44$, $p = 0.015$), shown in Table 3 and Fig. 1.

Discussion

There was no statistically significant difference between both groups ($p > 0.05$) regarding mean concentrations of zinc in plasma. It is worth noting that the plasma is a parameter of zinc that has a fast dynamic, constantly under homeostatic control, which may change in response to certain conditions, such as stress, infection, hormonal action, and food intake.^{21,22} These factors may have influenced the results of plasmatic zinc found in this study.

Another important aspect is that, in the bloodstream, 80% of zinc is present in the erythrocytes and only 16% is found in plasma. Since the half-life of erythrocytes is 120 days, erythrocytary zinc appears to be a parameter that assesses the nutritional status of this trace element with higher sensitivity.²³ Thus, unlike the results obtained in plasma, the mean values of erythrocytary zinc in the obese women were significantly lower than in the controls. The data are

Table 1 – Subjects characteristics.

Parameters	Obese women Mean ± SD	Control Mean ± SD	p
Age (years)	33.7 ± 7.89	31.2 ± 7.82	0.79
Weight (kg)	82.6* ± 11.40	54.4* ± 5.4	0.01
Height (cm)	154.3 ± 0.05	157.6 ± 0.06	0.52
BMI (kg/m ²)	34.5* ± 3.40	21.7* ± 1.90	0.001
WC (cm)	102.4* ± 8.50	75.4* ± 6.30	0.04

BMI, body mass index; WC, waist circumference.
* Values statistically significant between the obese women and the control group, Student's t- test ($p < 0.05$).

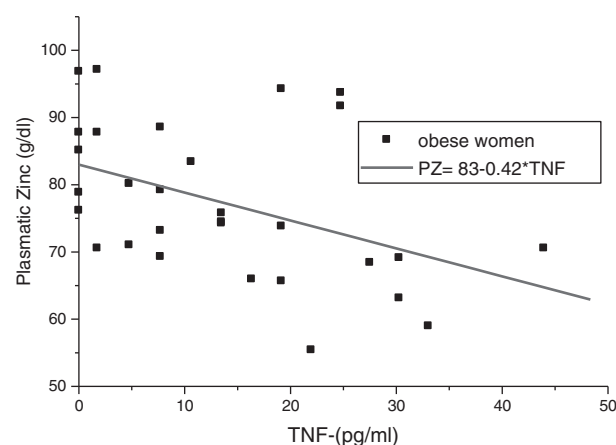


Fig. 1 – Correlation analysis between plasmatic zinc and concentration of TNF-α in plasma in obese women. n = 37, $r = -0.44$, $p = 0.015$.

Table 2 – Plasma and erythrocytary zinc, and plasma concentrations of IL-6 and of TNF- α . Values are means standard deviation.

Parameters	Obese women Mean \pm SD	Control Mean \pm SD	p
Plasma zinc ($\mu\text{g/dL}$)	72.2 \pm 9.0	73.4 \pm 8.5	0.48
Erythrocytary zinc ($\mu\text{g/gHb}$)	36.4 ^a \pm 15.0	45.4* \pm 14.3	0.025
IL-6 (pg/mL)	76.38 \pm 45.00	73.65 \pm 27.10	0.76
TNF- α (pg/mL)	42.02 ^b \pm 11.91	18.98 ^b \pm 0.97	0.001

IL-6, interleukin 6; TNF- α , tumor necrosis factor- α .

^a Values significantly different between the obese women and the control group, student T test ($p < 0.05$).

^b Values statistically significant between the obese women and the control group, Mann-Whitney test ($p < 0.05$).

Table 3 – Correlation analysis results for concentration of proinflammatory cytokines IL-6 and TNF- α in plasma and biochemical parameters of zinc in obese women.

Parameters	Plasma Zn		Erythrocytary Zn	
	Correlation coefficient (r)	p	Correlation coefficient (r)	p
IL-6 (pg/mL)	-0.30	0.082	0.11	0.526
TNF- α (pg/mL)	-0.44 ^a	0.015	-0.17	0.372

r, correlation coefficient; IL-6, interleukin 6; TNF- α , tumor necrosis factor- α ; Zn, zinc.

^a Values statistically significant between the obese women and the control group, student T test ($p < 0.05$).

consistent with those found by Marreiro, Fisberg, Cozzolino²⁴ and by Ozata et al.²⁵

The compartmentalization and metabolism of zinc in obesity have been extensively investigated. In the study by Begin-Heick et al.,²⁶ elevated zinc concentrations were found in the adipose tissue, liver, and muscle of obese mice. According to the authors, high concentrations of hormones, such as glucocorticoids, stimulate the synthesis of metallothionein, a protein that acts in the transportation and retention of zinc in some tissues.

Some mechanisms responsible for the compartmentalization of zinc in obese individuals are reported, such as the influence of zinc-transporter proteins and of the inflammatory process. Schmidt et al.²⁷ assessed the expression of zinc-transporter proteins, from the Zips and ZnTs families, in visceral and subcutaneous adipose tissue of obese patients, and it was observed that most of these proteins are expressed at a higher level in subcutaneous adipose tissue. According to the authors, the high gene expression of zinc-transporter proteins suggests that obesity can influence the system that transports this mineral in adipose tissue, favoring the manifestation of hypozincemia in obese individuals. However, the possible mechanisms involved are still unclear.

Some studies indicate the involvement of pro-inflammatory cytokines secreted by adipocytes in alterations often found in the metabolic behavior of zinc in obese patients. It is currently understood that adipose tissue, in addition to its function of storing energy reserves in the form of triglycerides, is an endocrine organ capable of producing several hormones and signaling molecules known as adipocytokines or adipokines. IL-6, IL-8, and TNF- α , secreted by adipose tissue, are molecules that have pro-inflammatory function, and are found in high concentrations in obese subjects.^{1,28}

The mean concentrations of pro-inflammatory adipokine IL-6 in plasma did not have a statistically significant difference

between groups. However, Piva et al.²⁹ found elevated concentrations of IL-6 in obese patients. The discrepancy observed in these results can be attributed to the sensitivity in the protocol used to analyze these cytokines.

Unlike the results of IL-6, the plasma concentrations of TNF- α in the obese women were higher than in the control group. These results are similar to those found by Park et al.³⁰ and by Winkler et al.³¹ in obese patients. The impact of the secretion of proinflammatory adipokines in adipocytes on metabolic disorders associated with obesity (such as insulin resistance, heart diseases, and liver diseases) has been widely demonstrated.

To better understand zinc metabolism in obese patients, the influence of inflammatory markers on the concentration of zinc in plasma was investigated. Although the plasmatic zinc was not different between the groups, there was a significant negative correlation between the concentrations of TNF- α in plasma and plasmatic zinc ($r = -0.44$, $p = 0.015$). According to Cousins et al.,⁷ TNF- α is a proinflammatory cytokine capable of stimulating the expression of encoding genes for zinc-transporter protein, such as Zip-14 and Zip-6, which contribute to the influx of zinc to the cells and specific tissues, causing a reduction of this mineral in blood components.

Other studies that investigated the existence of a negative association between pro-inflammatory markers and biochemical parameters of zinc suggest that this mineral is an important tool in the control of the expression of inflammatory cytokines. Zinc supplementation stimulates the synthesis of A-20 protein, which, in turn, contributes to reducing the activation of the signaling pathway of nuclear factor kappa β , preventing the synthesis and secretion of TNF- α and other pro-inflammatory cytokines.^{32,33}

Considering the complexity of the mechanisms involved in the changes of the behavior of mineral metabolism in obese patients, and the likely influence of pro-inflammatory adipokines in these alterations, further studies on the role of

these molecules in the distribution of zinc in obese individuals may contribute to a better understanding of the issue.

It is important to highlight the specificity and sensitivity of the kits used to analyze the inflammatory cytokines IL-6 and TNF- α , which certainly compromised the consistency of the data in this study. This discussion is also based on the absence of a homeostatic control of these substances, because the synthesis of cytokines is influenced by several factors, such as acute or chronic subclinical inflammation, which probably influenced the results found in this study.

Conclusion

The obese women evaluated in this study presented lower erythrocytic zinc levels than the control group. Furthermore, the analysis of the correlation between pro-inflammatory cytokines and biochemical parameters of zinc only achieved statistical significance in relation to TNF- α - this molecule has a negative correlation with plasmatic zinc. This result suggests a probable influence of the inflammatory process on the metabolism of zinc in obese patients. However, the inconsistent results of studies on the subject reinforce the need for further studies that may elucidate the mechanisms involved in metabolic aspects of zinc in obesity.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

- Bulló M, Casas-Agustench P, Amigó-Correig P, Aranceta J, Salas-Salvadó J. Inflammation, obesity and comorbidities: the role of diet. *Public Health Nutr.* 2007;10:1164-72.
- Shah A, Mehta N, Reilly MP. Adipose inflammation, insulin resistance, and cardiovascular disease. *J Parenter Enteral Nutr.* 2008;32:638-44.
- Whitaker KW, Totoki K, Reyes TM. Metabolic adaptations to early life protein restriction differ by offspring sex and post-warning diet in the mouse. *Nutr Metab Cardiovasc Dis.* 2011;23:345-56.
- World Health Organization (WHO). Global strategy on diet, physical activity and health: obesity and overweight. Geneva: WHO; 2007.
- Fett CA, Fett WCR, Padovan GJ, Marchini JS. Mudanças no estilo de vida e fatores de risco para doenças crônicas não transmissíveis e sistema imune de mulheres sedentárias. *Rev Nutr.* 2009;22:245-55.
- Kennedy ML, Failla ML. Zinc metabolism in genetically obese (ob/ob) mice. *J Nutr.* 1987;117:886-93.
- Cousins RJ, Liuzzi JP, Lichten LA. Mammalian zinc transport, trafficking, and signals. *J Biol Chem.* 2006;281:24085-9.
- Foster M, Samman S. Zinc and regulation of inflammatory cytokines: implications for cardiometabolic disease. *Nutrients.* 2012;4:676-94.
- Besecker BY, Exline MC, Hollyfield J, Phillips G, Disilvestro RA, Wewers MD, et al. A comparison of zinc metabolism, inflammation, and disease severity in critically ill infected and noninfected adults early after intensive care unit admission. *Am J Clin Nutr.* 2011;93:1356-64.
- Egefjord L, Jensen JL, Bang-Berthelsen CH, Petersen AB, Smidt K, Schmitz O, et al. Zinc transporter gene expression is regulated by pro-inflammatory cytokines: a potential role for zinc transporters in beta-cell apoptosis? *BMC Endocr Disord.* 2009;9:7.
- Liuzzi JP, Aydemir F, Nam H, Knutson MD, Cousins RJ. Zip 14 (SLC39a14) mediates non-transferrin-bound iron uptake into cells. *Proc Natl Acad Sci USA.* 2006;103:13612-7.
- Gaetke LM, McClain CJ, Talwalkar RT, Shedlofsky SI. Effects of endotoxin on zinc metabolism in human volunteers. *Am J Physiol.* 1997;272:E952-6.
- Aranha LN, Lobo JC, Stockler-Pinto MB, Leal VD, Torres JP, Mafra D. Relationship between zinc levels and plasma leptin in hemodialysis patients. *J Trace Elem Med Biol.* 2012;26:238-42.
- Ferro FE, de Sousa Lima VB, Soares NR, de Sousa Almondes KG, Pires LV, Cozzolino SM, et al. Parameters of metabolic syndrome and its relationship with zincemia and activities of superoxide dismutase and glutathione peroxidase in obese women. *Biol Trace Elem Res.* 2011;143:787-93.
- World Health Organization (WHO). Obesity: Preventing and managing the global epidemic. Geneva: WHO; 2000 (Technical report series n. 894).
- Rodríguez MP, Nazarino A, Demczyklo V, Cid A. A simpler method for the determination of zinc human plasma levels by flame atomic absorption spectrophotometry. *At Spectrosc.* 1989;10:68-70.
- Whitehouse RC, Prasad AS, Rabbani PI, Cossack ZT. Zinc in plasma, neutrophils, lymphocytes, and erythrocytes as determined by flameless atomic absorption spectrophotometry. *Clin Chem.* 1982;28:475-80.
- Van Assendelft OW. The measurement of hemoglobin. In: Izak G, Lewis SM, editors. *Modern concepts in hematology.* New York(NY): Academic; 1972. p. 14-25.
- Gibson RS. Assessment of trace element status. In: Gibson RS, editor. *Principles of nutritional.* New York: Oxford University Press USA; 1990. p. 511-76.
- Guthrie HA, Picciano MF. Micronutrient minerals. In: Guthrie HA. *Human nutrition.* New York; 1994. p. 351-7.
- Hambidge M. Biomarkers of trace mineral intake status. *J Nutr.* 2003;133 Suppl 3:S948-55.
- Hess SY, Peerson JM, King JC, Brown KH. Use of serum zinc concentration as an indicator of population zinc status. *Food Nutr Bull.* 2007;28 3 Suppl:S403-29.
- Davis CD, Milne DB, Nielsen FH. Changes in dietary zinc and copper affect zinc-status indicators of postmenopausal women, notably, extracellular superoxide dismutase and amyloid precursor proteins. *Am J Clin Nutr.* 2000;71:781-8.
- Marreiro DN, Fisberg M, Cozzolino SMF. Zinc nutritional status in obese children and adolescents. *Biol Trace Elem Res.* 2002;86:107-22.
- Ozata M, Mergen M, Oktenli C, Aydin A, Sanisoglu SY, Bolu E, et al. Increased oxidative stress and hypozincemia in male obesity. *Clin Biochem.* 2002;35:627-31.
- Begin-Heick N, Dalpe-Scott M, Rowe J, Heick HM. Zinc supplementation attenuates insulin secretory activity in pancreatic islets of the ob/ob mouse. *Diabetes.* 1985;34:179-84.
- Schmidt K, Pedersen SB, Brock B, Schmitz O, Fisker S, Bendix J, et al. Zinc-transporter genes in human visceral and subcutaneous adipocytes: lean versus obese. *Mol Cell Endocrinol.* 2007;264:68-73.
- Chudek J, Wiecek A. Adipose tissue, inflammation and endothelial dysfunction. *Pharmacol Rep.* 2006;58:81-8.
- Piva SJ, Tatsch E, De Carvalho JA, Bochi GV, Kober H, Duarte T, et al. Assessment of Inflammatory and oxidative biomarkers

- in obesity and their associations with body mass index. *Inflammation*. 2013;36:226-31.
30. Park HS, Park JY, Yu R. Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-alpha and IL-6. *Diabetes Res Clin Pract*. 2005;69:29-35.
31. Winkler G, Kiss S, Keszthelyi L, Sapi Z, Ory I, Salamon F, et al. Expression of tumor necrosis factor (TNF-alfa) protein in the subcutaneous and visceral adipose tissue in correlation with adipocyte cell volume, serum TNF-alfa, soluble serum TNF-receptor-2 concentrations and C-peptide level. *J Endocrinol*. 2003;149:129-35.
32. Prasad AS, Beck FW, Bao B, Fitzgerald JT, Snell DC, Steinberg JD, et al. Zinc supplementation decreases incidence of infections in the elderly: effect on zinc on generation of cytokines and oxidative stress. *J Clin Nutr*. 2007;85: 837-44.
33. Prasad AS. Zinc in human health: Effect of zinc on immune cells. *Mol Med*. 2008;14:353-7.