

Original article

Effect of Low Selenium Diet on Glutathione Peroxidase 3 Concentration in Male Sprague-Dawley Rats' Serum

Nikolina Kolobaric¹, Ana Stupin^{1,2}, Petar Susnjara¹, Lidija Baric¹, Anita Matic¹

¹ Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

² Faculty of Dental Medicine and Health in Osijek, Josip Juraj Strossmayer University of Osijek, Croatia

Corresponding author: Anita Matic, anita.cosic@mefos.hr

Abstract

Aim: Determination of antioxidative enzyme glutathione peroxidase 3 (GPx3) serum concentrations after consumption of food which contains different concentrations of selenium (Se).

Research subjects and methods: Four-week-old Sprague Dawley rats consumed food containing different concentrations of Se (food Divan) over a period of 10 weeks. The animals were divided into two groups: 1) normal Se (0.363 mg/kg Se) and 2) low Se (0.030 mg/kg Se). Each animal was weighed at the end of protocol, and serum samples were collected for determining GPx3 concentrations. All experimental procedures were in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and approved by the Ethics Committee of the Faculty of Medicine in Osijek and the Ministry of Agriculture of the Republic of Croatia.

Results: Different concentrations of Se in food did not cause a change in body weight. Food containing the recommended intake of Se according to the guidelines of the World Health Organization significantly increased GPx3 enzyme concentration (13.96 ± 0.42 mg/ml) when compared to low selective Se (12.04 ± 0.33 mg/ml, $p = 0.002$).

Conclusion: Serum concentration of the antioxidant enzyme GPx3 depends on the concentration of Se in food. It is shown that, in comparison with food with low Se levels, food containing a normal concentration of Se is enriched with the antioxidant GPx3 which, according to numerous studies, has a protective role in the human body.

(Kolobaric N, Stupin A, Susnjara P, Baric L, Matic A. Effect of Low Selenium Diet on Glutathione Peroxidase 3 Concentration in Male Sprague-Dawley Rats' Serum. SEEMEDJ 2019; 3(1): 50-59)

Received: March 30, 2019; revised version accepted: May 7, 2019; published: May 31, 2019

KEYWORDS: antioxidative enzymes, glutathione peroxidase 3, Se, Sprague Dawley rats

Introduction

The most important antioxidant enzymes are catalase, glutathione peroxidase, glutathione reductase and superoxide dismutase, which have harmful oxidative metabolic intermediates. These enzymes require cofactors selenium (Se), zinc, copper, iron, and magnesium for their catalytic activity (1).

Glutathione peroxidase (GPx), which is the subject of our study, is a family of enzymes that metabolize hydrogen peroxide and lipid hydroperoxide into water. They have a binding site for Se, which oxidizes in reaction with hydrogen peroxide (2-4). Glutathione peroxidase is involved in protection against oxidative stress in that it participates in the transfer of amino acids through the plasma membrane, removes the hydroxyl radical and the singlet oxygen, thereby detoxifying the hydrogen peroxide and the lipid peroxide by the catalytic action of GPx. It can replenish the active forms of the most essential vitamins, i.e., vitamins C and E (5).

It is known that oxidative stress is one of the pathogenic mechanisms that cause disorders of the vascular system and contribute to development and progression of various cardiometabolic diseases (hypertension, diabetes, atherosclerosis, obesity). A diet enriched with trace elements, which increase the concentration of antioxidant enzymes, could provide better protection against oxidative stress (6).

As part of GPx, Se is important for the regulation of the oxidative system (7-9). It is an essential trace element which must be ingested in sufficient quantity through food. In many countries, there is malnutrition due to the lack of Se as a micronutrient. Inadequate Se intake causes many different disorders (necrotizing cardiomyopathy, peripheral myopathy, reduced muscle tone, concentration problems, hair loss and nail splitting) (10-12). In our previous animal study, we showed that low dietary Se content affects the function of aorta, reduces ACh-induced relaxation, which is dominantly

mediated by NO, and also increases the level of local oxidative stress (13).

Human studies have shown that Se concentrations are inversely related to mortality and occurrence of cancer (14, 15). Se functions as an antimutagenic agent that prevents the transformation of healthy cells into malignant ones; it is assumed that these protective effects are primarily associated with the activity of GPx (16, 17).

Glutathione peroxidase 3 (GPx3) is the only enzyme in the GPx group that functions in extracellular space. The substrates for this enzyme are hydrogen peroxide and phospholipid hydroperoxides, which play a significant role in the antioxidative processes in the blood (18) in that they decrease oxidative stress by reducing H₂O₂ and organic hydroperoxides to their corresponding alcohols and oxygen (19). Since increased GPx3 activity is found in certain subtypes of tumors, like ovarian tumors (20), it can also be used as a biomarker.

This study aims to determine the level of the GPx3 antioxidative enzyme in serum samples with different levels of Se and to determine whether Se causes a change in body mass.

Materials and Methods

Ethical Approval

Experimental procedures complied with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe No 123, Strasbourg 1986) and were approved by the Ethics Committee of the Faculty of Medicine, University of Osijek (Class: 602-04/14-08/06, No: 2158-61-07-14-05), and authorized by the Ministry of Agriculture of the Republic of Croatia (Class: UP/I-322-01/14-01/90, No: 525-10/0255-15-4). The tests were performed in the Laboratory for Vascular Physiology and Laboratory for Molecular and Clinical Immunology at the Department of Physiology and Immunology of the Faculty of Medicine Osijek, Croatia.

Southeastern European Medical Journal, 2019; 3(1)

Experimental groups

Food was prepared at the Faculty of Agrobiotechnical Sciences Osijek, Croatia, based on the wheat briquettes recipe developed by Mucedola, Italy. Wheat was specially grown in soil with different concentrations of selenium and zinc, so that the concentration of said micronutrients was adjusted according to the requirements of the experiment. Four-week-old healthy male Sprague Dawley rats (N = 7 per group) consumed the above-mentioned food for ten weeks. The animals were randomly divided into groups:

- 1) normal Se group (0.363 mg/kg Se) and
- 2) low Se group (0.030 mg/kg Se).

Rats were housed doubly in shoebox-style cages with free access to food and tap water, housed in a temperature of -21°C-22°C, humidity- and light-controlled room, maintained on a 12:12 hour light : dark cycle.

Determination of Se (normal or low concentration) in food is consistent with previous studies (13, 21-24).

After 10 weeks of feeding, the animals were weighed and then anesthetized using a combination of ketanest S 75 mg/kg (Ketanest S 25 mg/ml, 2 ml ampoules, Pfizer) and midazolam 0.5 mg/kg (Midazolam Torrex 5 mg/ml, 3 ml, Torrex Chiesi Pharma). Blood samples were collected immediately after decapitation (arterial and venous blood) in empty tubes without anticoagulants to obtain serum and centrifuged at 3500 rpm for 10 minutes. The separated serums were stored in a refrigerator at -80 ° C until analysis.

Glutathione peroxidase 3 (GPx3) determination in serum samples

GPx3 concentrations were determined using the commercially available enzyme immunoassay kit purchased from LifeSpan BioSciences, USA (LSBio Cat. No. LS-F6289). Each well of the supplied microtiter plate was precoated with an antibody. First, 100 μ l of serum samples and

standards (in duplicates) were put in their appropriate place on the ELISA plate and incubated for 1 hour at 37 ° C. After the first incubation, the unbound standard or sample was washed away, 100 μ l of biotin-conjugated detection antibody was added to each well and the same time and temperature incubation was continued. After that, it was washed 3 times and Avidin-Horseradish Peroxidase (HRP) conjugate was added, followed by a 30-minute incubation at 37 ° C. A TMB substrate was then added, which reacted with the HRP enzyme, resulting in color development. At the end of the protocol, stop solution was added to terminate the color development reaction and optical density (OD) was measured at a wavelength of 450 nm on the PR 3100 TSC Microplate Reader in the Laboratory for Molecular and Clinical Immunology at the Department of Physiology and Immunology of the Faculty of Medicine, Josip Juraj Strossmayer University of Osijek.

Statistical analysis

Differences in normally distributed numerical variables between groups were tested with the Student t-test, and in case of deviations from normal distribution with the Mann-Whitney U test (SigmaPlot version 11.2, Systat Software, Inc., Chicago, USA) The level of significance was determined at $p < 0.05$. The sample size was determined using the Sigma Plot version 11.0 program. For the power of the test of 0.8, p value less than 0.05 and the minimum expected difference of 0.25, it was found that at least 4 animals per group were required. Results are presented below as the mean \pm standard deviation (SD).

Results

Effect of diet on body weight

There was no difference in body weight between the normal Se and the low Se group ($p < 0.05$) (Figure 1).

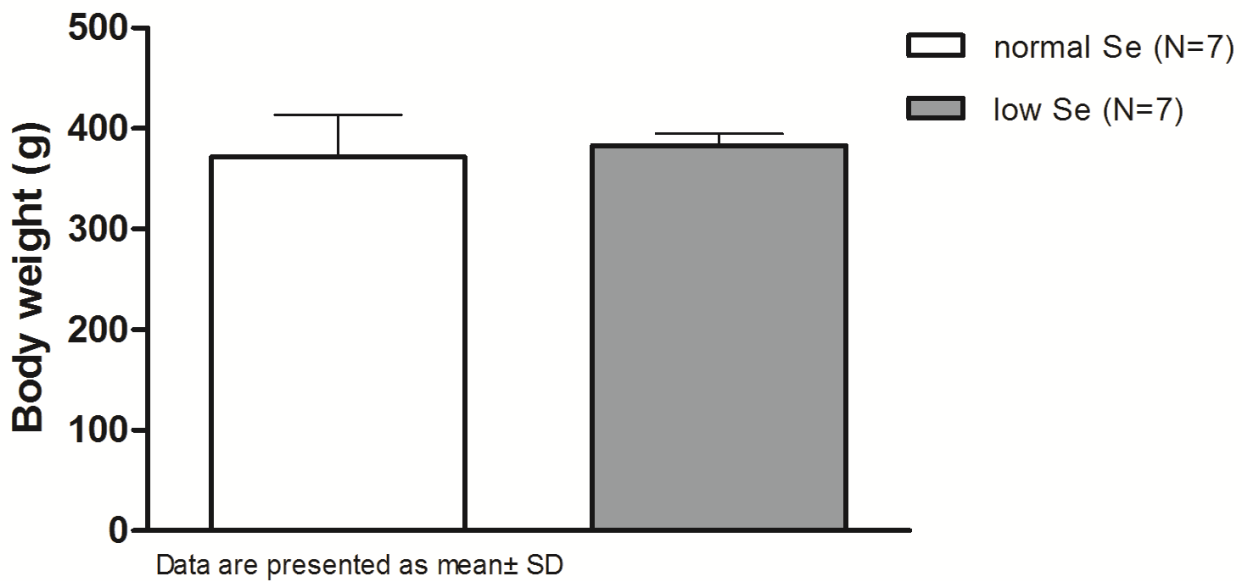


Figure 1. Body weight in experimental rat groups

GPx3 serum concentration

The group of animals that consumed food with normal Se content had significantly increased

GPx3 enzyme concentrations (13.96 ± 1.11 ng/ml) compared to the low selective Se content group (12.04 ± 0.89 ng/ml, $p = 0.002$) (Figure 2).

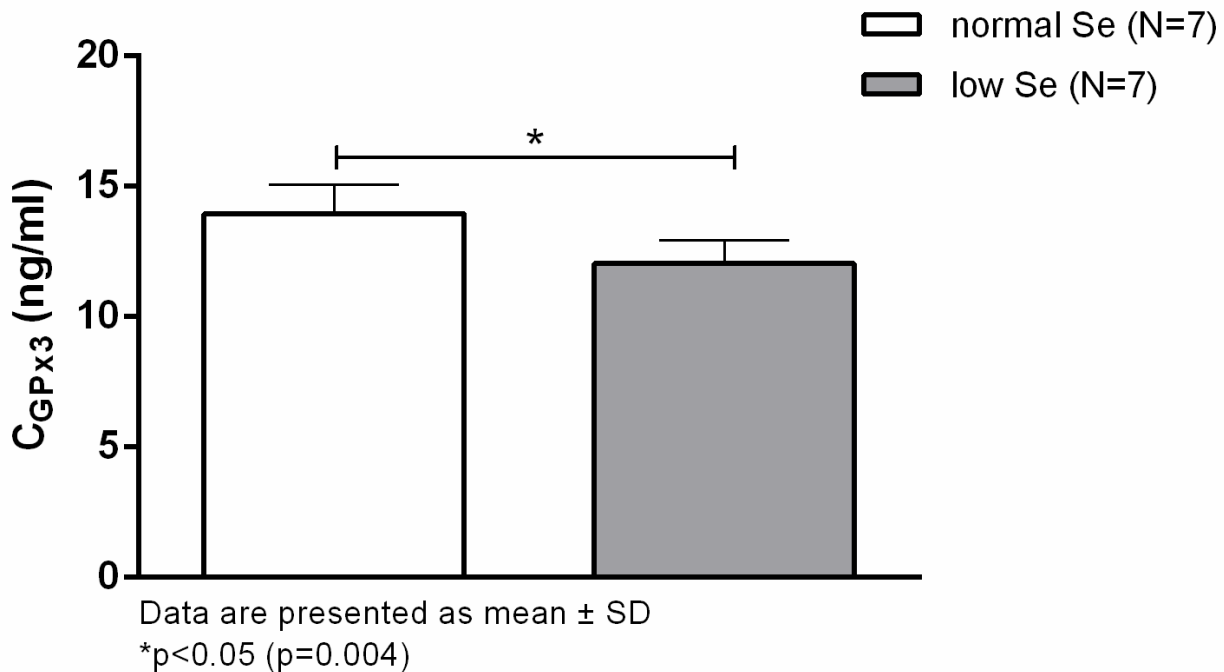


Figure 2. Concentration of the antioxidative enzyme GPx3 (ng/ml) in serum samples

Discussion

We recently showed that low dietary Se content increases oxidative stress, which is possibly caused by decreased gene expression of the antioxidant enzyme GPx1 in thoracic aortic tissue without changes of the same enzyme in serum samples (13). Gene expression of other antioxidative enzymes (Cu/Zn SOD and catalase) did not change significantly. GPx3 is a better isoform of GPx for determination of the antioxidant effect of Se in blood samples, so we decided to measure the more appropriate GPx isoform, which is readily detectable in the blood (25).

The main findings of this study are the following: a) serum concentration of the antioxidant enzyme GPx3 depends on the concentration of Se in food; b) our study once again confirmed that food with sufficient levels of Se (according to the World Health Organization (WHO) and the American Institute of Nutrition (AIN)) is richest with the antioxidant GPx3, which has a protective role in the body; c) different concentrations of Se have no effect on body weight.

Trace elements are essential for living organisms because they are necessary for normal function of the organism, its growth and performance of its metabolic functions. Amounts in the human body range from 1.5 mg to 4.5 g. It is characteristic for trace elements that very small amounts of a trace element affect the condition of the entire organism (26, 27). Levels of Se in food can vary in different parts of the world and countries (28, 29). Intakes of Se are high in America and Japan, and much lower in Europe, particularly Eastern Europe (30).

It was recently shown that Se has an important role in antioxidant selenoproteins used for protection against oxidative stress initiated by ROS and its compounds in different types of cancers, cardiovascular diseases, immune system diseases and aging (31, 32). The World Health Organization published findings that a diet which contains 0.1 mg Se per kilogram of food is sufficient for normal function of the organism (33), while the American Institute of

Nutrition (AIN) and the TestDiet® AIN-93 Growth Purified Diet indicate that 0.24 mg Se/kg in food is optimal (21). According to these guidelines, we determined the values of low (0.030 mg/kg Se) and normal (0.363 mg/kg Se) levels of Se for our research. Measuring Se levels in blood samples seems to be a good indicator because plasma and serum contain about 75% of the Se. The level of Se found in these samples is directly related to recent dietary intakes (29).

Our research has shown that consuming food containing sufficient amounts of Se according to the guidelines provides a significantly higher level of GPx3 antioxidants in plasma compared to foods with low Se concentration (Figure 2). Such findings indicate that Se is directly responsible for increasing the antioxidative status of GPx and for lowering oxidative stress.

Se and its protective role against disease are mostly related to the removal of free radicals and the enzymatic breakdown of oxygen metabolites (34). For example, in tuberculosis patients, reduced oxidative stress was caused by ROS generation with Se supplementation (35). Of all types of tissue, the thyroid gland has the highest Se concentration, and in the form of GPx3, Se protects thyroid cells from free radicals and oxidative stress (36, 37). It was shown that in Hashimoto's thyroiditis Se supplementation significantly lowered thyroid peroxidase autoantibody titer after 3 months (38). Furthermore, studies showed that Se has a beneficial effect on lowering the risk for different types of cancer (39- 43). It can reduce oxidative damage and prevent DNA damage (44). It can be used as alternative medicine by cancer patients undergoing radiotherapy (45 - 47). In addition, cellular and molecular processes that might be involved in the anti-cancer effects of Se are stabilization of the immune response, induction of programmed cell death and inhibition of angiogenesis (44).

Lack of the Se causes irreversible brain injury (48). Similar, lower serum Se was also found in children with epileptic seizures or febrile seizures and in adults with epileptic seizures (49, 50).

Se has also been proved to be important for maintaining the immune system. It has been shown that Se in a concentration of 400 μ g per day significantly increased the number of T-cells (51), and supplementation with 100 μ g per day for 6 months significantly increased the response to antigen challenge (52). Se deficiency may cause a slower immune response because of the negative influence it has on immune cells (53).

ROS is involved in regulating the synthesis of adhesion molecules on endothelial cells, and is essential for inflammatory responses during the early stages of a disease (54, 55). The expression of intercellular and vascular adhesion molecules increases significantly during inflammation and is involved in the firm adhesion of leukocytes and endothelial cells (54). Se nutritional status can directly influence vascular endothelial cell functions, e.g., under low Se conditions, aortic endothelial cells exhibit increased platelet activating factor biosynthesis, which causes vascular disorders that are affected by increased oxidative stress (56). It was also found that the production of prostaglandins was significantly decreased in Se-deficient endothelial cells, which are associated with the

pathophysiology of several inflammatory diseases through significantly increased biosynthesis of thromboxane B₂ (TXB₂) and 15-hydroperoxyeicosatetraenoic acid (15-HPETE) (57, 58). Crucial role of Se in oxidative stress and endothelial influence has been demonstrated by the study Stupin et al. (2017), which showed that increased oxidative stress affects a NO-mediated response in low-Se aortas probably due to decreased NO bioavailability (13). Selenoproteins regulate vascular tone by establishing a balance between superoxide anion and nitrogen oxide (7), which was found to be the case in this study as well.

Numerous studies have shown the important role that Se plays in different health/disease states. Further research is necessary to make use of the benefits of this important element as much as possible. Observing the specific role of Se in the endothelium, glutathione peroxidase was found to have an indispensable role in the maintenance of normal vascular function. Specifically, low Se decreased tissue expression of GPx1 and blood concentration of GPx3, which caused an increase in oxidative stress and disrupted endothelial function (Figure 3).

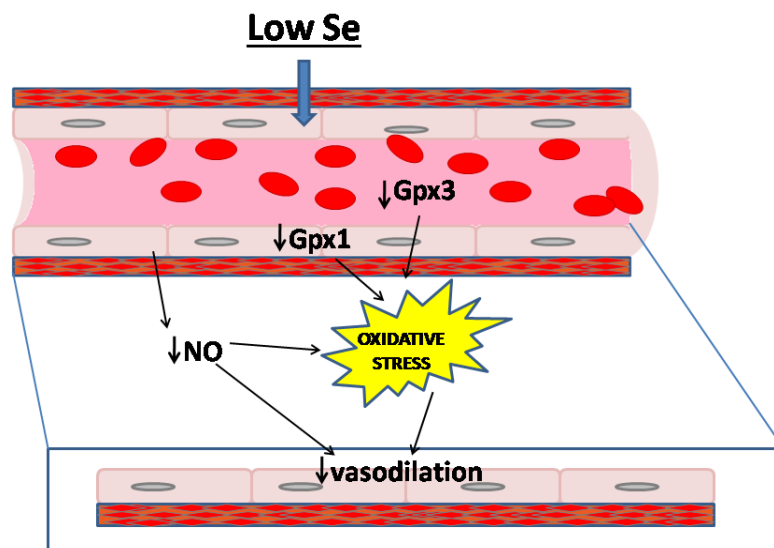


Figure 3. Negative effect of low Se levels on changed vascular antioxidant status and consequent endothelial dysfunction

Sources of Funding

This study was supported by grants from the Ministry of Science, Education and Sports of the Republic of Croatia, #219-2160133-2034 and #079-0790462-0450; the Cro-Hu bilateral project "Common pathways for microcirculatory dysfunction in diabetes mellitus and hypertension" (2009–2011); and the Norwegian Ministry of Foreign Affairs Program in Higher

Education, Research and Development HERD/Agriculture project "Cooperation in education and research on mineral improved crop production for human and animal health in Balkan Countries" (number 09/1548).

References

1. Remacle J, Michiels C, Raes M. The importance of antioxidant enzymes in cellular aging and degeneration. *EXS*. 1992;62:99-108.
2. Brigelius-Flohé R, Maiorino M. Glutathione peroxidases. *Biochim Biophys Acta*. 2013 May;1830(5):3289-303.
3. Veerareddy S, Cooke CL, Baker PN, Davidge ST. Gender differences in myogenic tone in the superoxide dismutase knockout mouse: an animal model of oxidative stress. *Am J Physiol. Am J Physiol Heart Circ Physiol*. 2004;287(1):H40-5
4. Brigelius-Flohe R. Glutathione peroxidases and redox-regulated transcription factors. *Biol Chem* 2006;387(10-11):1329-35.
5. Hatfield DL, Gladyshev VN. How Se has altered our understanding of the genetic code. *Mol Cell Biol* 2002;22:3565-76.
6. Brigelius-Flohe R. Tissue-specific functions of individual glutathione peroxidases. *Free Radic Biol Med*. 1999;27(9-10):951-65.
7. Brigelius-Flohé R, Banning A, Schnurr K. Se-dependent enzymes in endothelial cell function. *Antioxid. Redox Signal*. 2003; 5, 205–215.
8. Ghadirian P, Maisonneuve P, Perret C, Kennedy G, Boyle P, Krewski D et al. A case-control study of toenail Se and cancer of the breast, colon, and prostate. *Cancer Detect Prev* 2000;24(4):305-13.
9. Cooper GM, Hausman RE. Stanica: Molekularni pristup. Medicinska naklada, Zagreb 2010
10. Mates JM, Perez-Gomez C, Nunez De Castro I. Antioxidant enzymes and human diseases. *Clin Biochem* 1999;32:595–603.
11. Harissa GL. Oxidative Stress and Paraoxonase Activity in Experimental Selenosis: Effects of Betaine Administration. *Biol Trace Elem Res*. 2013;152(2):258-66.
12. Guyton AC, Hall JE. Medicinska fiziologija 12 izdanje; Medicinska naklada d.o.o 2012, Zagreb
13. Stupin A, Ćosić A, Novak S, Vesel M, Jukić I, Popović B, Karalić K, Lončarić Z, Drenjančević I. Reduced dietary Se impairs vascular function by increasing oxidative stress in Sprague-Dawley rat aortas. *International Journal of Environmental Research and Public Health*. 14 (2017), 6; 591-591
14. Black RE. Zinc deficiency, infectious disease and mortality in the developing world. *J Nutr* 2003; 133: 1485S-9S
15. Brown KM, Arthur JR. Se, selenoproteins and human health: a

- review. *Public Health Nutr* 2001; 4:593-9
16. Friedmann Angeli JP, Conrad M. Se and GPX4, a vital symbiosis. *Free Radic Biol Med*. 2018; S0891-5849(18)30104-7.
 17. Swart R, Schutte AE, van Rooyen JM, Mels CMC. Serum Se levels, the selenoprotein glutathione peroxidase and vascular protection: The SABPA study. *Food Res Int*. 2018;104:69-76.
 18. Davis CD, Uthus EO. Dietary folate and Se affect dimethylhydrazine-induced aberrant crypt formation, global DNA methylation and one carbon metabolism rats. *J Nutr* 2003;133(9):2907-14.
 19. Loscalzo, J. Nitric oxide insufficiency, platelet activation, and arterial thrombosis. *Circ. Res*. 2001;88, 756-762.
 20. Schwarz K, Foltz CM. Se as an integral part of factor 3 against dietary necrotic liver degeneration. *J. Am. Chem. Soc*. 1957; 79, 3292-3293.
 21. Reeves P. Components of the AIN-93 Diets as Improvements in the AIN-76A Diet. *J. Nutr*. 1997;127, 838S-841S.
 22. Cakmak I. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant Soil* 2008;302, 1-17.
 23. Reilly C. *Se in Food and Health*. Springer Science & Business Media, Berlin/Heidelberg, Germany, 2006; p. 9.
 24. Lyons GH, Lewis J, Lorimer MF, Holloway RE, Brace DM, Stangoulis JCR, Graham RD. High-Se wheat: Agronomic biofortification strategies to improve human nutrition. *Food Agric. Environ*. 2004; 2, 171-178.
 25. Oh IJ, Kim HE, Song SY, Na KJ, Kim KS, Kim YC, Lee SW. Diagnostic value of serum glutathione peroxidase 3 levels in patients with lung cancer. *Thorac Cancer*. 2014. 5(5):425-30.
 26. Čvorišćec D, Čepelak I. *Molekularna dijagnostika; Štrausova Medicinska biokemija*. Medicinska naklada Zagreb, 2009.
 27. Zhang XW. My Element: Selenium. *Chem. Eur. J*. 2019;25:2649-2650.
 28. Tinggi U, Reilly C, Patterson CM. Determination of Se in foodstuffs using spectrofluorometry and hydride generation atomic absorption spectrometry. *J Food Comp Anal*. 1992; 5:269-80.
 29. Reilly C. *Se in Food and Health*; Springer Science & Business Media: Berlin/Heidelberg, Germany, 2006; p. 9.
 30. Rayman MP. Se and human health. *Lancet*. 2012; 31:379(9822):1256-68.
 31. Tinggi U. Se: Its Role as Antioxidant in Human Health. *Environmental Health and Preventive Medicine* 13.2 (2008): 102-108.
 32. Cai Z, Zhang J, Li H. Selenium, aging and aging-related diseases. *Aging. Clin. Exp. Res*. 2018
 33. World Health Organization (WHO). *Trace Elements in Human Nutrition and Health; Se*; WHO: Geneva, Switzerland, 1996; pp. 105-122.
 34. Mugesh G, Singh HB. Synthetic organoSe compounds as antioxidants: Glutathione peroxidase activity. *Chemical Society Reviews*. 2000; 29, 347-357.
 35. Seyedrezazadeh E, Ostadrahimi A, Mahboob S, Assadi Y, Ghaemmagami J, Pourmogaddam M. Effect of vitamin E and Se supplementation on oxidative stress status in pulmonary tuberculosis patients. *Respirology*. 2008; 13, 294-298.

36. Schomburg L, Köhrle J. On the importance of Se and iodine metabolism for thyroid hormone biosynthesis and human health. *Mol Nutr Food Res* 2008; 52: 1235–46.
37. Ventura M, Melo M, Carrilho F. Selenium and thyroid disease: From pathophysiology to treatment. *Int. J. Endocrinol.* 2017
38. Toulis KA, Anastasilakis AD, Tzellos TG, Goulis DG, Kouvelas D. Se supplementation in the treatment of Hashimoto's thyroiditis: a systematic review and a meta-analysis. *Thyroid* 2010; 20: 1163–73.
39. Zhuo H, Smith AH, Steinmaus C. Se and lung cancer: a quantitative analysis of heterogeneity in the current epidemiological literature. *Cancer Epidemiol Biomarkers Prev* 2004; 13: 771–78
40. Amaral AF, Cantor KP, Silverman DT, Malats N. Se and bladder cancer risk: a meta-analysis. *Cancer Epidemiol Biomarkers Prev.* 2010; 19: 2407–15.
41. Peters U, Takata Y. Se and the prevention of prostate and colorectal cancer. *Mol Nutr Food Res.* 2008; 52: 1261–72.
42. Hatfield DL, Carlson BA, Tsuji PA, Tobe R, Gladyshev VN. Selenium and cancer. In *Molecular, Genetic, and Nutritional Aspects of Major and Trace Minerals.* Academic. Press. 2017;463–473
43. Kieliszek M, Lipinski B, Błażej S. Application of sodium selenite in the prevention and treatment of cancers. *Cells.* 2017;6:39
44. Rayman MP. Se in cancer prevention: a review of the evidence and mechanism of action. *Proc Nutr Soc.* 2005; 64(4):527–42.
45. Ponholzer A, Struhal G, Madersbacher S. Frequent use of complementary medicine by prostate cancer patients. *Eur Urol.* 2003; 43(6):604–8.
46. Micke O, Buntzel J, Kisters K, Schafer U, Micke P, Mucke R. Complementary and alternative medicine in lung cancer patients: a neglected phenomenon? *Front Radiat Ther Oncol.* 2010; 42():198–205.
47. Song M, Kumaran MN, Gounder M, Gibbon DG, Nieves-Neira W, Vaidya A, Hellmann M, Kane MP, Buckley B, Shih W, et al. Phase I trial of selenium plus chemotherapy in gynecologic cancers. *Gynecol. Oncol.* 2018;150:478–486
48. Burk RF, Hill KE. Selenoprotein P-expression, functions, and roles in mammals. *Biochim Biophys Acta* 2009; 1790: 1441–47
49. Ashrafi MR, Shams S, Nouri MMohseni M, Shabani R, Yekaninejad MS et al. A probable causative factor for an old problem: Se and glutathione peroxidase appear to play important roles in epilepsy pathogenesis. *Epilepsia* 2007; 48: 1750–55.
50. Amiri M, Farzin L, Moassesi ME, Sajadi F. Serum trace element levels in febrile convulsion. *Biol Trace Elem Res* 2010; 135: 38–44.
51. Wood SM, Beckham C, Yosioka A, Darban H, Watson RR. β -Carotene and Se supplementation enhances immune response in aged humans. *Integr Med* 2000; 2: 85–92.
52. Rayman MP. The importance of Se to human health. *Lancet.* 2000; 356: 233–41
53. Peter R. Hoffmann and Marla J. Berry. The influence of Se on immune responses. *Mol Nutr Food Res.* 2008; 52(11): 1273–1280.

54. Kvietys PR, Granger DN. Role of reactive oxygen and nitrogen species in the vascular responses to inflammation. *Free Radical Biology & Medicine*. 2012; 1;52(3):556-592.
55. Wolin MS. Reactive oxygen species and the control of vascular function. *Am J Physiol Heart Circ Physiol*. 2009;296(3):H539-49.
56. Corl CM, Cao YZ, Cohen ZS, Sordillo LM. Oxidant stress enhances Lyso-PAF-AcT activity by modifying phospholipase D and phosphatidic acid in aortic endothelial cells. *Biochem Biophys Res Commun*. 2003; 14;302(3):610-4.
57. Cao YZ, Reddy CC, Sordillo LM. Altered eicosanoid biosynthesis in Se-deficient endothelial cells. *Free Radic Biol Med*. 2000; 1;28(3):381-9.
58. Weaver JA, Maddox JF, Cao YZ, Mullarky IK, Sordillo LM. Increased 15-HPETE production decreases prostacyclin synthase activity during oxidant stress in aortic endothelial cells. *Free Radic Biol Med*. 2001; 1;30(3):299-308.