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# EFFECT OF INADEQUATE SELENIUM INTAKE ON THE CHANGES OF AORTIC WALL IN YOUNG NORMOTENSIVE RATS

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**ABSTRACT**

The aim of this study is to investigate the influence of inadequate selenium (Se) intake on the changes of aortic wall in young normotensive rats. Twenty male Wistar rats, 8 weeks old, separated into 2 groups, were used. During 8 weeks one group had low Se diet (LSe) and the second - adequate Se diet (NSe). The serum concentration of Se, and serum level of anti-elastin and anti- tropoelastin antibodies were measured. Immunofluorescence expression of glutathione peroxidase-1 (GPx-1) at aortic wall was influenced proportionally by the different Se intake. The histological examination revealed that rats with low Se intake had alterations of aortic walls. Morphometry of aortic walls evaluated higher thickness of thoracic and abdominal aorta in rats from LSe group than the NSe group ($p = 0.0001$). A relatively weak inverse relationship between the serum Se concentration and the thickness of aortic wall was obtained ($r= -0.42$; $p=0.04$). The serum level of anti-elastin antibodies increased under inadequate Se intake ($p=0.03$).

Low GPx -1 expression in aortic wall due to inadequate Se intake caused pathological changes of aortic wall and elastin turnover of young normotensive rats that may cause hemodynamic disturbances in the future.

**Key words**: selenium, aortic wall, elastin turnover, Wistar rats

## BACKGROUND

It is described that the physiological effects of selenium (Se) occur mainly through the functions of selenoproteins. Glutathione peroxidases (GPx) and thioredoxin reductases are the main selenoproteins expressed in endothelial cells. GPx-1 is the most abundant intracellular isoform of the GPx antioxidant enzyme family that catalyzes reduction of hydrogen peroxide ($H_2O_2$) and organic peroxides and plays the most important role for their elimination from the cells (1, 2). $H_2O_2$ modulates the different aspects of the function of endothelial cells: their growth and proliferation; apoptosis; endothelium-dependent vasodilatation; barrier function; endothelial inflammatory answer; endothelial control on vessel's remodeling (3, 4). Endothelial cells are continuously exposed to a blood flow and are the primary target of an oxidant-induced injury. Tissue expression of selenoproteins depends on daily Se intake. It has been established that a diet containing 0.1 μg Se/g of food is enough for normal growth and reproduction in mammals (5, 6).

Elastin is the main component of the vessel wall. Pathological changes of an elastin turnover and production of a collagen may lead to a disturbance of the vessels, increase a stiffness of the vessel walls and decrease a blood supply to the organs. Serum level of antibodies to tropoelastin (ATEABs) and to α-elastin (AEABs) correlated with the production and breakdown respectively of the elastic tissue. The ratio between ATEABs and AEABs may give information for the elastin

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metabolism and the alterations of the vessels, because of close relation between the peptides and a presence of specific antibodies (7).

**The aim** of this investigation is to evaluate the changes of aortic wall in young normotensive rats under diet of inadequate selenium content.

## MATERIALS AND METHODS

The experiment was performed in accordance with the Animal Welfare Act Regulations and was approved by University Ethic s Committee.

- Twenty male 8-weeks old Wistar (WKY) rats, divided into two groups, had received low (0.05μg Se/ g of food), assigned as LSe and adequate (0.11μg Se/ g of food) – NSe (control group), selenium content diets during 8 weeks.
- Their systolic blood pressure (SBP) was measured indirectly using a tail cuff method, before and after applied diets.
- The rats were placed in the single chambers and had water and food ad libitum. Daily intake of food was checked for each rat.
- At the age of 16-weeks, under anesthesia of Pentobarbital Sodium (40 mg/kg i.p.), a blood was taken from the abdominal aorta and a serum was separated.
- The serum Se concentration was determined at Central Clinical Laboratory of University Hospital "Alexandrovska", Sofia, using flameless atomic absorption spectrophotometric analysis.
- The aortas of the rats, separated into 2 parts – thoracic and abdominal, were extirpated. Longitudinal slices were prepared and stained by Hemalaun-Eosin (H&E). The morphological changes were examined and described under light microscope. A measurement of the thickness of aortic walls of each rat was performed at 10 points (including the thickest and the thinnest areas), using an ocular micrometer at the Laboratory of Department of Common and Clinical Pathology, MU – Pleven.
- Imunofluorescence cell staining was performed at the Immunohistological laboratory of Institute of Biology and Immunology of Reproduction of Bulgarian Academy of Sciences – Sofia, using kits and protocols of Santa Cruz Biotechnology, Inc. USA - [home page of Internet]; c2007-08. Available from http://www.scbt.com/protocol_immunofluorescence_cell_staining.html.

After deparaffinization and hydratation of slides we used:

▪ 10% normal blocking serum (normal donkey sera: sc-2044) for 20 minutes to suppress non-specific binding of IgG,

▪ Incubation with primary antibody (GPx-1 /H-19/ goat polyclonal antibody: sc-22146) at dilution range 1:100 for 60 minutes,

▪ Slides were incubated in fluorochrome conjugated secondary antibody (donkey anti-goat IgG-FITC: sc-2024) at dilution range 1:200 for 45 minutes,

▪ Cover slips were mounted with Ultra Cruz mounting medium (sc-24941) and examined under a laser scanning co focal microscope "Leica TCS SPE", 405 nm exiting length of light; Acs APO 40.0 x 15; immersing objective.

▪ Photos were taken and the results compared, using semi qualitative analysis of changes.

- AEABs were determined by the method of indirect home-made enzyme-linked immunosorbent assay (ELISA) in the Immunological Laboratory of the Biology Department of Medical University, Pleven. The plates (Greiner Microlon, Monroe, NC, USA) were coated with 100 ml rat lung a-elastin (Elastin Products Company, Owensville, MO, USA) (10 mg/mL) for two hours at room temperature and over the course of one night at 48C. After washing with phosphate-buffered saline ю 0.05% Tween-20 (PBS–Tween), the wells were blocked with 100 mL 0.1% bovine serum albumin in PBS–Tween-20 for one hour at 378C. The tested serum samples (100 mL) diluted 1:5 were dropped, and incubated for one hour at $37^0$C. After washing, 100 mL anti-rat IgG peroxidase conjugate (Sigma-Aldrich, St Louis, MO, USA), at a dilution of 1:6400, were added. Incubation was performed for one hour at $37^0$C. After washing and incubation with substrate solution (0.8 mg/mL in 0.05 mol/L citrate buffer, rO 5.0, containing 0.01% $H_2O_2$), the reaction was stopped at the 20th minute by 50 mL 8N $H_2SO_4$. The absorptions were read at 492 nm by an automated ELISA reader. The following controls were used: (1) substrate control: assay buffer only, washing solutions and substrate added to the polystyrene wells coated with antigen a-elastin; (2) detection antibody control: detection antibody added

directly to the wells coated with antigen; (3) negative control to assess the specificity of the reaction: antigen replaced by human albumin solution and serum samples; and the (4) positive control: the tested sample replaced by antibodies to rat a-elastin in assay buffer. All samples were analyzed in triplicates and averaged. Pig tropoelastin is used for determination of ATEABs by the method of indirect ELISA as the same manner.

- The F-test of Fisher (ANOVA), multiple range tests (LSD) and regression analysis were used for data analysis. The tests were performed with statistical program SPSS, v. 15.

**RESULTS**

No significant differences between groups for measured systolic blood pressure were observed after applied diets. Rats from LSe group had SBP 117±5mm Hg, and these from NSe group - 114±5mm Hg.

The serum Se concentration was significantly different in the investigated groups in dependence of Se content of the applied diet. For the LSe group serum Se as mean±SE was 499±37µg/L and 599±37µg/L for the control group **(Figure 1).**

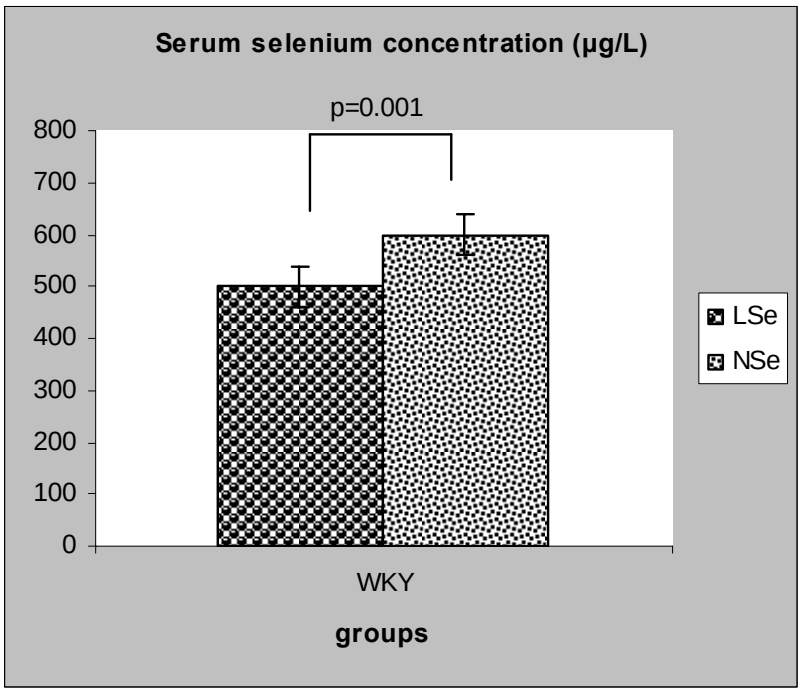


**Figure 1.** Serum selenium concentration of experimental groups after applied diets with different selenium content as means±SE (µg/L).

Higher thickness of aortic wall of rats from the group received low Se diet than this of control group was determined **(Figure 2)**.

A relatively weak inverse relationship between the serum Se concentration and the thickness of aortic wall was obtained: for thoracic aorta (r= -0.42; p=0.04) and for abdominal aorta (r= -0.38; p=0.06).

The histological examinations showed pathological changes of aortic walls of rats from group of Lse diet **(Figure 3)** - thickening of smooth muscle cells layer and roughness of endothelium and normal state in rats of the control group **(Figure 4)**. Immunofluorescence examination of GPx-1 revealed predominant expression in endothelium and weak localization in the medial smooth muscle cells. Fluorescence microscopy image analysis showed the different degree of fluorescent expression in rats on a diet of different Se content in accordance to serum Se concentration.

Low Se diet caused increase of elastin degradation – AEABs=0.471 as mean of OD in LSe group and – 0.253 in NSe group. Significant decrease of ratio between processes of synthesis and degradation of this main extracellular matrix protein of aortic wall was observed – ratio ATEABs / AEABs was 1.7 to 2.4 **(Figure 5).**

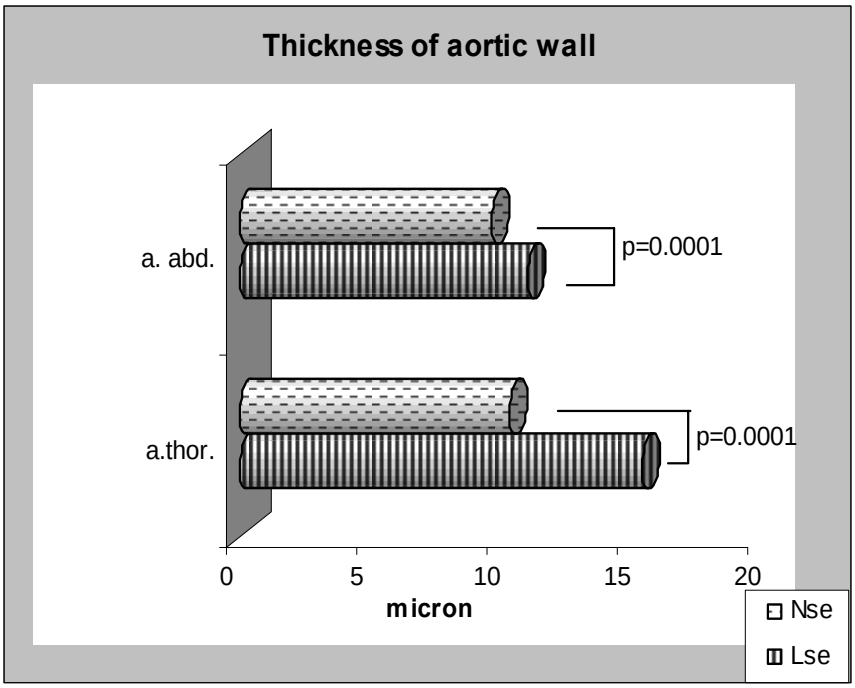


**Figure 2.** Thickness of thoracic and abdominal aorta walls of experimental groups as means (μm) after applied diets with different selenium content.

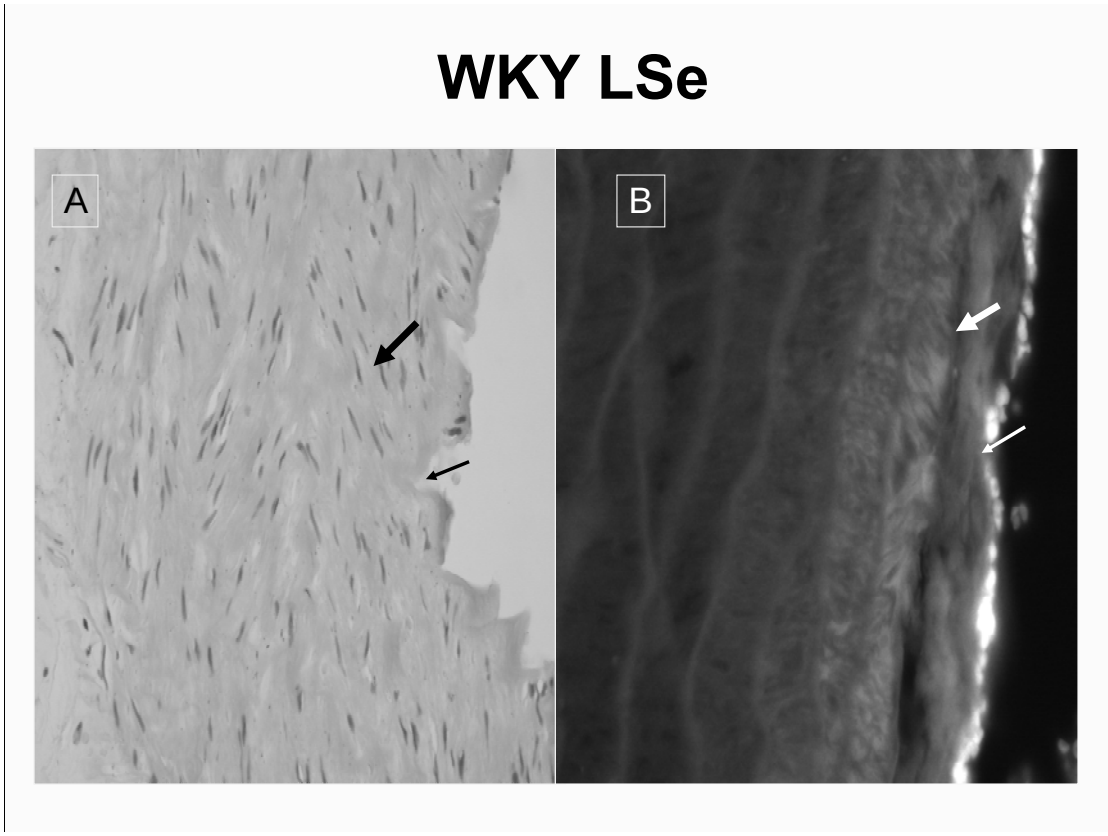


**Figure 3.** Representative photomicrograph of aortic wall (thin arrow – endothelium; thick arrow – media) of WKY rat under low Se diet.
A. Histology – thickening of smooth muscle cells layer and roughness of endothelium (H&E magnification x40);
B. Expression of GPx-1 – low intensity of immunofluorescence (magnification x40).

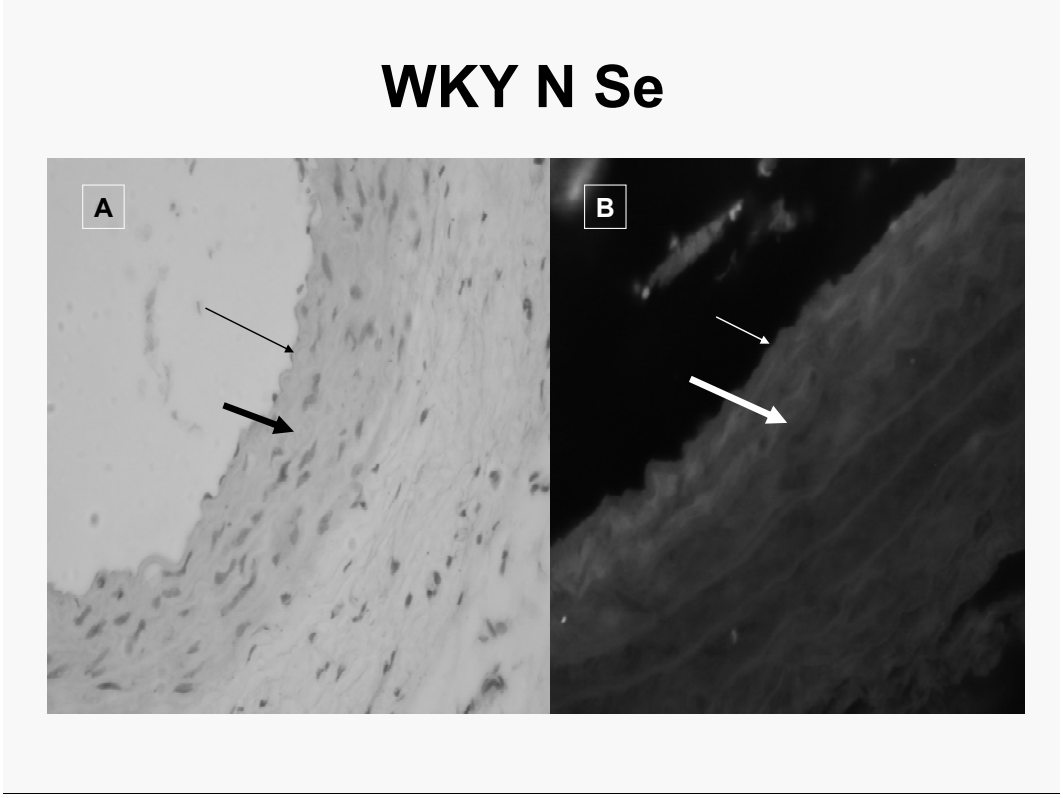


**Figure 4**. Representative photomicrograph of aortic wall (thin arrow – endothelium; thick arrow – media) of WKY rat under normal Se diet.
A. Histology – intact endothelium and normal thickness of the wall (H&E magnification x40); B. Expression of GPx-1 – moderate intensity of immunofluorescence (magnification x40).

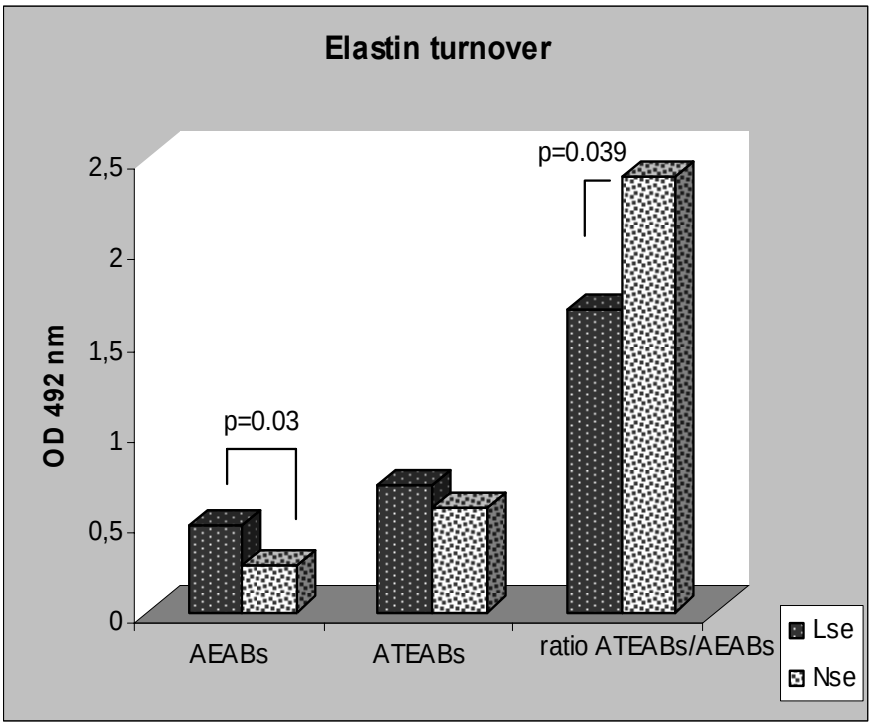


**Figure 5.** Anti-elastin antibodies (AEABs), Anti-tropoelastin antibodies (ATEABs) as means (OD 492 nm) and ratio between ATEABs/AEABs serum level in Wistar rats under different selenium diets.

**DISCUSSION**

The biological role of selenoproteins in mammals includes an optimal endocrine and immune function, prevention of the cardiovascular and neoplastic diseases. The selenoproteins are grouped into 3 families of enzymes: glutathione peroxidases, thioredoxin reductases, and iodothyronine $5^{I}$ deiodinases. Six isoenzymes of glutathione peroxidase that participate in antioxidative defense of mammalian organism have been evaluated. The thioredoxin reductases control cellular redox systems. The researchers have identified 3 iodothyronine deiodinases, which catalyze a release of iodine from 5 or $5^{I}$ position of the iodothyronin substrates and play an important role for activation and inactivation of the thyroid hormones in all tissues (1, 2).

Selenium has entered the organism mainly with food. The serum Se concentration was significantly decreased under diet of inadequate Se content. It was described that low Se intake decreased significantly GPx-1 activity. GPx-1 deficiency directly induces an increase of vascular oxidant stress, with resulting endothelial dysfunction (8, 9, 10).

Using animal models it was evaluated that abnormal redox status of aortic wall was in the main importance for development of arterial hypertention and atherosclerosis and had determining effect on the disturbed control of vasomotor tone. The endothelial dysfunction causes a dysbalance of the synthesis of a vasodilatator Nitric oxide (NO) and a vasoconstrictor endothelin-1 that increases the resistance of the vessels. The NO contributes to a vascular tone, preserves the endothelial integrity, and inhibits the smooth muscle cell migration and proliferation. The low GPx-1 activity decreases a bioavailable NO, increasing activity of arginase and increasing production of peroxinitrites. Endothelial dysfunction precedes development of vessel's lesions (8,11). The morphological data observed by our experiment showed that alterations of vessel walls began from thoracic aorta to abdominal aorta. Hypertrophic remodeling, in which the media thickens to encroach on the lumen results in increased media cross-sectional area. Our results shown, that low GPx-1 expression caused degenerative changes in aortic wall and its thickening, probably because of high presence of reactive oxygen species and low availability of NO. Determined relationship between the serum Se concentration and the thickness of aortic wall gave evidence that selenium is significant factor for normal state of aortic wall.

Elastic is the major component of the vessel wall and it is responsible for an elasticity of the big vessels. Elastin is synthesized and split continuously. Elastin peptides derived from its degradation are present in the circulating blood and they are a stimulus for increase production of AEABs. The significant changes of the elastin metabolism play a role on the alterations of the integrity of the rich of elastin organs - vessels, lung, skin, muscles (12, 13).

The turnover rate for collagen and elastin is low in healthy arteries, but vascular pathology upsets the regulatory pathways that maintain this balance. The increased oxidative stress of aortic wall accelerates degradation of elastic fibers leading to loss of arterial wall resilience. Their degradation products can act as signal transducers and regulate cellular proliferation, migration, phenotype and extracellular matrix (ECM) proteins degradation. The over-expression of both Pro-inflammatory and proteinase-inhibitory molecules dramatically increases arterial ECM proteins synthesis. In the same time elastin and elastic fibers are progressively degraded by enzymatic processes, involving an imbalance between proteases – elastin degrading enzymes, including several matrix metalloproteases (MMPs), such as MMP-2, MMP-9 and antiproteases - tissue inhibitors of metalloproteases (TIMPs). An imbalance between MMPs and TIMPs is linked to the degradation of the extracellular matrix associated with several physiologic and pathologic events including angiogenesis, invasion and metastasis. Many evidences suggest that TIMPs are multifunctional proteins, which regulate cell proliferation, apoptosis, proMMP-2 activation, and angiogenesis (14, 15, 16).

Our results support these findings. Low Se intake significantly increases elastin degraded products and das not significantly influence tropoelastin content of aortic wall thus significantly decreased the ratio between processes of elastin synthesis and elastin degradation. We suppose that low expression of selenoproteins in aortic wall and altered oxidative balance caused imbalance between proteases and antiproteases those resulted in

pathological changes of elastin turnover and vessel wall.

We speculate that inadequate Se intake in humans may cause also pathological changes of vessel walls and may affect normal functions of cardiovascular system. A low Se intake worsens the antioxidant status and is a risk factor for the development of cardiovascular diseases (CVD), (17,18). The cross-sectional and longitudinal population studies observed negative relation between systolic blood pressure and serum Se in humans. GPx-1 activity has been the strongest unvaried predictor of the risk for cardiovascular events and Se supplementation is important for secondary prevention of coronary heart disease (19, 20, 21).

## CONCLUSIONS

Low GPx -1 expression in aortic wall due to inadequate Se intake caused pathological changes of aortic wall and elastin turnover of young normotensive rats that may cause hemodynamic disturbances in the future. Adequate daily Se intake is important for maintenance of normal functional state of aortic wall.

We suggest that it is important to make more special clinical investigations for monitoring serum Se concentration and GPx-1 activity of whole blood of the humans lived at the areas with poor selenium soil to prevent early development of CVD.

## REFERENCES


1. Combs G.F.Jr. Selenium in global food systems. *Br. J. Nutr* , 85(5):517-547, 2001
2. Moghadoszadeh, B., Beggs, A. Selenoproteins and their impact on human health through diverse physiological pathways. *Physiology*, 21:307–315, 2006
3. Cai, H. Hydrogen peroxide regulation of endothelial function: origins, mechanisms, and consequences. *Cardiovasc Res,* 68:26–36, 2005
4. Faraci, Frank M. Hydrogen peroxide – watery fuel for change in vascular biology. *Arterioscler Thromb Vasc Biol*, 26:1931–1933, 2006
5. Panel on Dietary Antioxidants and Related Compounds, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of DRIs, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids.* DC: TheNational Academies Press, Washington, pp.284–324, 2000, See www.nap.edu/catalog/9810.html (last checked 11 January 2012)
6. Report of a Joint WHO/FAO Expert Consultation Diet. *Nutrition and Prevention of Chronic Diseases*. TRS 916, Geneva: WHO, 2003
7. Colburn, K., Sandberg, L., Malto, M., Roos, P., VanGent, C., Finley, J. Age-related circulating antibody levels to tropo- and alfa-elastin correlate with and are specific for their respective serum peptides in rats. *J Invest Med*, 43:284A, 1995
8. Forgione, M., Weiss, N., Heydrick, S., Cap, A., Kings, E., Bierl, C., Eberhardt, R., Farber, H., Loscalzo, J. Cellular glutathione peroxidase deficiency and endothelial dysfunction. *Am J Physiol Heart Circ Physiol,* 282:255–261, 2002
9. Driscoll, D., Copeland, P. Mechanism and regulation of selenoprotein synthesis. *Annu Rev Nutr,* 23:17–40, 2003
10. Daniels, L. A. Selenium: does selenium status have health outcomes beyond overt deficiency. *MJA,* 180(3):373-374, 2004
11. Thengchaisri, N., Travis, W., Wang, W., Xu, X., Li, Z., Fossum, T., Kuo, L. Upregulation of argnase by $H_2O_2$ impairs endothelium-dependent nitric oxide-mediated dilation of coronary arterioles. *Arterioscler Thromb Vasc Biol,* 26:2035–2042, 2006
12. Colburn, K., Langga-Shariffi, E., Kelly, G., Malto, M., Sandbeg, L., Baydanoff, S., Green, L. Abnormalities of serum antielastin antibodies in connective tissue diseases. *J Invest Med*, 51:104-9,2003
13. Colburn, K., Rambharose, J., Malto, M., Baydanoff, S., Sandbeg, L., Green, L. Abnormally low antibody markers of elastin synthesis in patients with Fibromyalgia syndrome. *J Musculoskel Pain*, 14:13–19, 2006
14. Fassina, G., Ferrari, N., Brigati, C., Benelli, R., Santi, L., Noonan, DM., Albini, A.Tissue inhibitors of metalloproteases: regulation and biological activities. *Clin Exp Metastastasis*, 18(2):111-120, 2000
15. Jiang, Y., Goldberg, ID., Shi, YE. Complex roles of tissue inhibitors of metalloproteinases in cancer. *Oncogene*, 21 (14):2245-2252, 2002

16. Jacob, M. Extracellular matrix remodeling and matrix metalloproteinases in the vascular wall during aging and in pathological conditions. *Biomed Pharmacother* , 57:195–202, 2003
17. Neve, J. Selenium as a risk factor for cardiovascular diseases. *J Cardiovasc Risk,* 3:42–47, 1996
18. Neve, J. New approaches to assess selenium status and requirement. *Nutr Rev*, 58(12):363-369, 2000
19. Blankenberg, S., Ruppercht, H., Bickel, C., Torzewski, M., Hafner, G., Tiret, L. Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. *N Engl J Med* , 349:1605–1613, 2003
20. De Lorgeril, M., Salen, P. Secondary prevention of coronary heart disease. *Asia Pac J Clin Nutr,* 14:84–91, 2005
21. Nawrot, T., Staessen, J., Roels, H., Hond, E., Thijs, L., Fagard, R., Dominiczak, A., Struijker-Boudier, H. Blood pressure and blood selenium: a cross sectional and longitudinal population study. *Eur Heart J*, 10:1093–1099, 2006