ПРОБЛЕМНАЯ СТАТЬЯ

NEW WAY TO ASSESS THE BIOELEMENT SELENIUM NUTRITIONAL STATUS NON INVASIVELY IN *VIVO*

J. Prejac[1], S. Morović[2], S. Drmić[3], J. Morović[4], Z. Pišl[5], B. Momčilović[6]

[1] University Hospital Centre Zagreb, Department of Oncology, Kišpatićeva 12, 10000 Zagreb, Croatia; e-mail: juraj.prejac@gmail.com

[2] Aviva Medical Center, Nemetova 2, 10000 Zagreb, Croatia; e-mail: Sandra.morovic@poliklinika.aviva

[3] Cliniical Hospital Dubrava, Department of Clinical Psychiatry, Avenija Gojka Šuška, 10000 Zagreb, Croatia; e-mail: stipe.drmic@gmail.com

[4] Centar IGW, d.o.o. Gestalt d.o.o., Kralja Držislava 12, 10000n Zagreb, Croatia; e-mail: jadran.morovic@zg-tcom.hr

[5] Institute for Research and Development of the Sustainable Ecosystems (IRES), Jagodno 100a, 10410 Velika Gorica, Croatia; e-mail: pisl@net.hr

[6] Institute for Research and Development of the Sustainable Ecosystems (IRES), Srebrnjak 59, 10000 Zagreb, Croatia; e-mail: berislav.momcilovic@gmail.com

ABSTRACT. Selenium (Se) is essential trace element in human nutrition. The aim of this study was to assess selenium nutritional status by analyzing Se frequency distribution in the long-term biological indicator tissue of hair (H·Se) and in the short-term biological indicator tissue of whole blood (WB·Se). Hair selenium was analyzed in 1073 apparently healthy adult Croats (339 ♂ and 734 ♀) and the whole blood selenium was analyzed in a random subsample of 91 ♂ and 143 ♀. Samples were analyzed by the ICP-MS at the Center for Biotic Medicine, Moscow, Russia. There were no significant gender dependent difference in the selenium adequate linear reference range which was ($\mu g \cdot g^{-1}$) for H·Se 0.078–0.701 and for WB·Se 0.120–0.200, respectively. Hair selenium concentrations below 0.078 and 0.120 for WB·Se indicate selenium deficiency. The estimated upper adequate selenium limits for H·Se and WB·Se are set at 0.701 and 0.200 $\mu g \cdot g^{-1}$, respectively. The linear segment of the generated H·Se logistic distribution sigmoid curve is considered to represent adequate Se intake range. This adequate selenium intake segment can be itself partitioned by a 60:30:10 percentage ratio into Sparsely adequate (♀ 0.078–0.405, ♂ 0.174–0.474), Adequate (Optimal) (♀ 0.405–0.573, ♂ 0.474–0.608), and Ample adequate segment (♀ 0.573–0.623, ♂ 0.608–0.709). These reference dose data are essential for continuous monitoring of the selenium nutritional status and selenium supplementation medication The noninvasive selenium status assessment is especially important when dealing with a vulnerable segments of human population like pregnant and lactating women, and their infants. The Median Derivatives Bioassay provides a new public health asset for general medical practice.

KEYWORDS: Selenium, Hair, Whole blood, Se nutritional status.

INTRODUCTION

Selenium (Se) is an essential trace bioelement in human health and nutrition (World Health Organization…, 1996; 2004). Selenium is ubiquitously present in the human diet and it's daily dietary intake varies in a wide range (in $\mu g \cdot d^{-1}$) from 30 in Finland, to 60–70 and/or 80 in the USA, about 105 in Turkey, and 113–120 in UK (Institute of Medicine…, 2000; Reilly, 2006; Reyman, 2012). Se is essential constituent of selenoprotein glutathione peroxidase (GSH) and its isomorphs; a cluster of enzymes which protects the cell membrane polyunsaturated fatty acids from the free radicals oxidative species (ROS) damage (Roman et al., 2013). Selenium, together with iodine, is crucial in the control of the thyroid gland hormone synthesis, and it is also essential for normal metabolism, growth and development (Berry, Larsen, 1992).

A fatal congestive heart failure condition of severe dietary selenium intake deficiency (30–50

* Corresponding author:
Berislav Momčilović
E-mail: berislav.momcilovic@gmail.com

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$\mu g \cdot g^{-1} \cdot d^{-1}$), when associated with the Coxackie viral infection, is known as a Keshan disease (Germelli et al., 2002), whereas dietary selenium doses exceeding >900 $\mu g \cdot g^{-1}$ d^{-1} and when associated with the plasma Se levels >1000 $\mu g \cdot L^{-1}$, generates selenosis, a toxic syndrome of dermatitis, lose hair, diseased nails, and peripheral neuropathy (Yang et al., 1983). Whether the higher, i.e. higher than optimally abundant or mildly excessive dietary Se intakes, may prevent cancer, is still controversial (Griffin, 1979; Bera et al., 2012; Cai et al., 2016).

Dietary selenium intake ranges widely from 7 μg per day to 4990 μg per day, and the UK recommendations for selenium intake average are 60 μg per day for men and 53 μg per day for women (Reyman, 2012). Similar expert consensus Recommended Dietary Allowances (RDA) for selenium in the USA are set at 55 and 45 $\mu g \cdot g^{-1}$ for adults of both sexes aged 19–50 y and over 50 y, respectively (Institute of Medicine…, 2000). Only recently, the safe upper levels of Se dietary intake are set at 450 μg per day (Expert Group on Vitamins and Minerals…, 2003).

Assessing selenium requirements and adequacy of its nutritional status is still the challenging issue (Thomson, 2004). Thus far, serum and/or plasma selenium and their selenoproteins were the mostly tested bio indicators (Kipp et al., 2015), i.e., the indicators under the strong homeostatic control. The aim of this study is to demonstrate that the Se nutritional status may be accurately assessed by analyzing its cumulative frequency distribution in the long term biological indicator tissue of hair (H·Se) and in contrast to that of the short term biological indicator tissue of whole blood (WB·Se). Median derivatives bioassay was used to analyze the collected data (Momčilović, et al., 2017).

SUBJECTS AND METHODS

This prospective, observational, cross-sectional, and exploratory nutritional epidemiology study was approved by the Ethical Committee of the Institute for Research and Development of the Sustainable Eco Systems (IRES), Zagreb, Croatia. The study was conducted by adherence to the Declaration of Helsinki on Human Subject Research (Browne, 2005). Every subject gave her/his written consent to participate in the study and filled out a short questionnaire on his/her health status and medical history (data not shown) (Oppenheim, 2004). None of the tested subjects used selenium containing hair shampoo.

Hair selenium (H·Se) was analyzed in a random sample of 1073 apparently healthy adults (339 men, 734 women). Whole blood was analyzed in a random subset of 212 subjects (143 women and 91 men); the median age of women and men was 47 and 50 years, respectively. Our population consisted of subjects from the general Croatian population who were interested to learn about their health status; the majority of them were living in the capital city region of Zagreb, Croatia. All the subjects consumed their usual home-prepared mixed mid-European diet, and none of them have reported an adverse medical health condition.

Hair selenium (H·Se) and whole blood selenium (WB·Se) were analyzed with the inductively coupled plasma mass spectrometry (ICP-MS) (Elan 9000, Perkin Elmer, USA) at the Center for Biotic Medicine (CBM) laboratory in Moscow, Russia (Appendix A). The CBM is an ISO Europe certified commercial laboratory for analyzing bio elements, i.e., electrolytes, trace elements, and ultra-trace elements, in different biological matrices. CBM laboratory is also a member of the exclusive External Quality Assessment of Surrey scientific group for the quality control of the trace element analysis in UK.

Preparation of scalp hair and whole blood samples for selenium ICP-MS analysis followed the International Atomic Energy Agency and other relevant recommendations (International Atomic Energy Agency (IAEA)…, 1980; Burges, 2000) (Appendix B). The detection limits for hair selenium (H·Se) and whole blood selenium (WB·Se) were ($\mu g \cdot g^{-1}$) 0.0001 and 0.00105 $\mu g \cdot g^{-1}$, respectively. All chemicals were of pro analysis grade (Khimmed Sintez, Moscow, Russia). Selenium belongs to the pleiad of 125 elements sharing the same mass number (number of isotopes/name of the element): 4 Zn, 7 Ga, 13 Ge, 16 As, 22 Se, 18 Br, 17 Kr, 11 Rb, 10 Sr, 6 Y, 1 Zr (Momčilović et al., 2008).

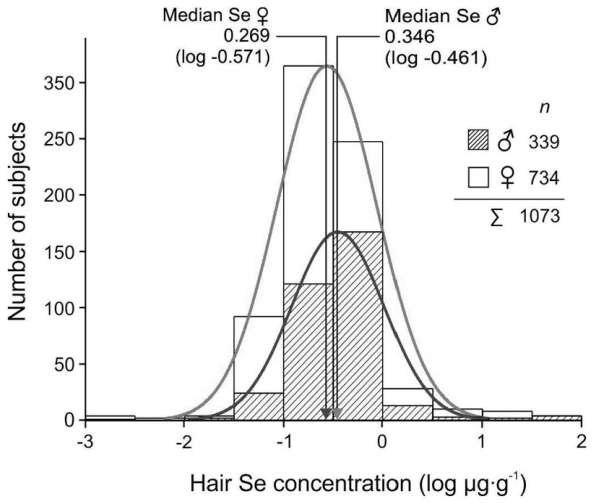
Median derivatives bioassay (Appendix C). The frequency distribution of selenium in the hair and whole blood samples was analyzed with the median derivatives bioassay of the log transformed data what yielded Gaussian bell-shaped frequency distribution. The data were fitted to form the sigmoid logistic regression function (power function) for men and women separately (Finey, 1952):

$$A2 + \frac{A1 - A2}{1 + \left(\frac{x}{x0}\right)^{p}},$$

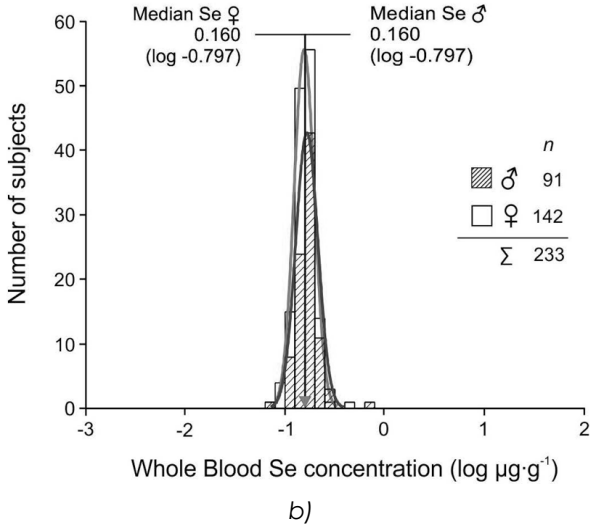
where $A1$ is the initial value (lower horizontal asymptote), $A2$ is the final value (upper horizontal asymptote), $x0$ is the center (point of inflection) of the detected median ($M0$), p is power (the parameter that affects the slope of the area about the inflection point).

The OriginPro 8.0 data analysis and graphing software was used for this analysis (OriginLab Corp., OriginPro Version 8.0., Northhampton, MA). We can visually describe the sigmoid frequency distribution curve into five distinct segments describing Se nutritional status: (1) Deficient Se nutritional status (Lower horizontal asymptote), (2) the lower boundary segment connecting the Deficient and the adequate linear segment, (3) Adequate linear segment, (4) the upper boundary segment connecting the Adequate linear segment with the Excessive upper horizontal asymptote (5).

The Adequate linear segment of the sigmoid bioassay curve can be further partitioned by a 60:30:10 ratio to differentiate between the respective Sparsely adequate, Adequate (Optimal), and Amply adequate Se nutritional status. This partition of Adequate linear segment into three referent clinical intervals (Hermann, 1971), allows us to monitor the range where Se supplementation would be beneficiary, the range where Se supplementation is unnecessary and the range where Se supplementation may induce adverse health effects, respectively.



a)



b)

Fig. 1. *Hair selenium frequency distribution in men and women (a) and whole blood selenium frequency distribution in men and women (b)*

RESULTS

Selenium was detected in all the 1073 analyzed hair samples and in every of the 234 analyzed whole blood sub-samples, respectively. After the data were log transformed, the previous skewed and kurtic selenium data distribution assumed a standard bell shaped Gaussian frequency distribution curve for both the hair and whole blood (Fig. 1).

Hair selenium median derivatives (Appendix C) were used to fit the bioassay sigmoid power function curve. The data on the upward and downward arm of the median derivatives H·Se and WB·Se are shown in Fig. 2 for men and women, respectively.

The bioassay sigmoid curve showed that there is a linear segment of median derivatives bioassay that covered the same ranges of ♀d2-u2 and ♂D2-U2 for both H·Se and WB·Se, respectively.

This linear range represents the adequate (optimal) selenium nutritional range where the rate of hair and whole blood selenium incorporation (saturation) is linearly proportional to the dietary selenium intake. This Adequate H·Se range of concentrations of Croatian women have a span from 0.078–0.623 $\mu g \cdot g^{-1}$ (median 0.269 $\mu g \cdot g^{-1}$) and that for Croatian men ranged from 0.134–0.701 $\mu g \cdot g^{-1}$ (median 0.346 $\mu g \cdot g^{-1}$). The respective low linear region of the sigmoid power function curve below d2 for women and D2 for men were defined as a deficient Se nutritional status. Similarly, the respective upper linear region of the sigmoid power function curve above the respective linear segments u2 for women (range u3–u6) and segment U2 for men (range U3–U6) were defined as an excessively high H·Se exposure. Evidently, on average, men tend to retain somewhat more Se in their hair than women, whereas this linear WB·Se segment was identical for both women and men, i.e., their Coefficient Intervals were completely merged.

Hair selenium levels below 0.070 ($\mu g \cdot g^{-1}$) for women and 0.134 ($\mu g \cdot g^{-1}$) for men, indicate selenium nutritional deficiency, whereas H·Se above 0.623 and 0.701 should be considered excessive for ♀ and ♂, respectively. Similarly, our WB·Se deficiency level is below 130 in ♂ and 120 in ♀ whereas the WB·Se excessive levels are 200 in ♂ and 193 in ♀, respectively.

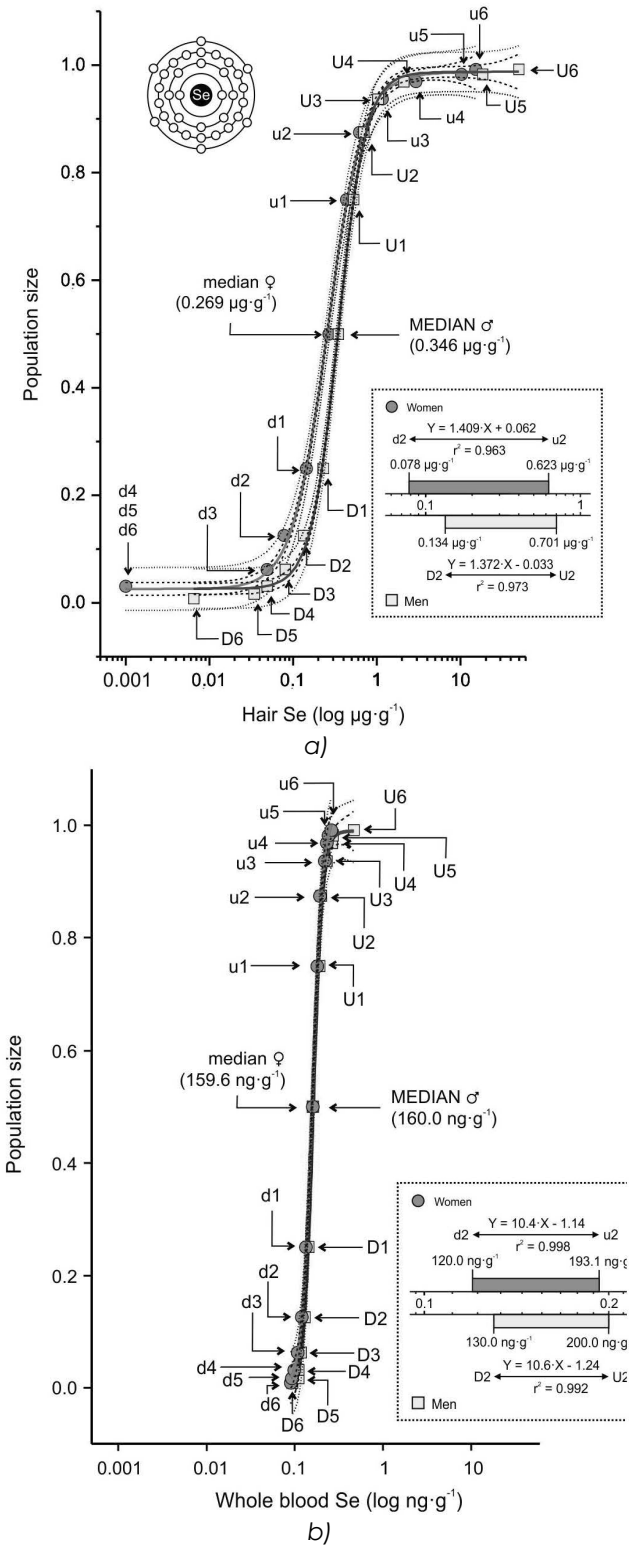


Fig. 2. Selenium median derivatives bioassay (D, U Men downward (D) and upward (U) median derivatives; d, u Women downward (d) and upward (u) median derivatives. See Appendix C for median derivatives input numerical values):
a – hair: the difference between the H·Se median derivatives of men n=339 (■) and women n=734.
Logistic function Y = A2+ (A1-A2)/(1 + (X/X0)ᵖ), - - - 0.95 confidence limit, ••• 0.95 prediction limit.
Men: Y = 0.991 + (0.026 – 0.991)/(1 + (X/0.029)^1.640), r² = 0.998; Women: Y = 1.006 + (0.021 – 1.006)/(1 + (X/0.027)^1.661), r² = 0.999.
Box: Hair selenium linear saturation range for ♂ and ♀ (log concentration);
b – whole blood: the difference between the WB·Se median derivatives of men n=91 (■) and women n=143 (○).
Logistic function Y = = A2 + (A1-A2)/(1 + (X/X0)ᵖ), - - - 0.95 confidence limit, ••• 0.95 prediction limit.
Men: Y = 0.992 + (0.013 – 0.992)/(1 + + (X/4.075)^12.145), r² = 0.999; Women: Y = 0.989 + (0.016 – 0.989)/(1 + (X/3.867)^12.288), r² = 0.999. Box: Whole blood selenium linear saturation range for ♂ and ♀ (log concentration)

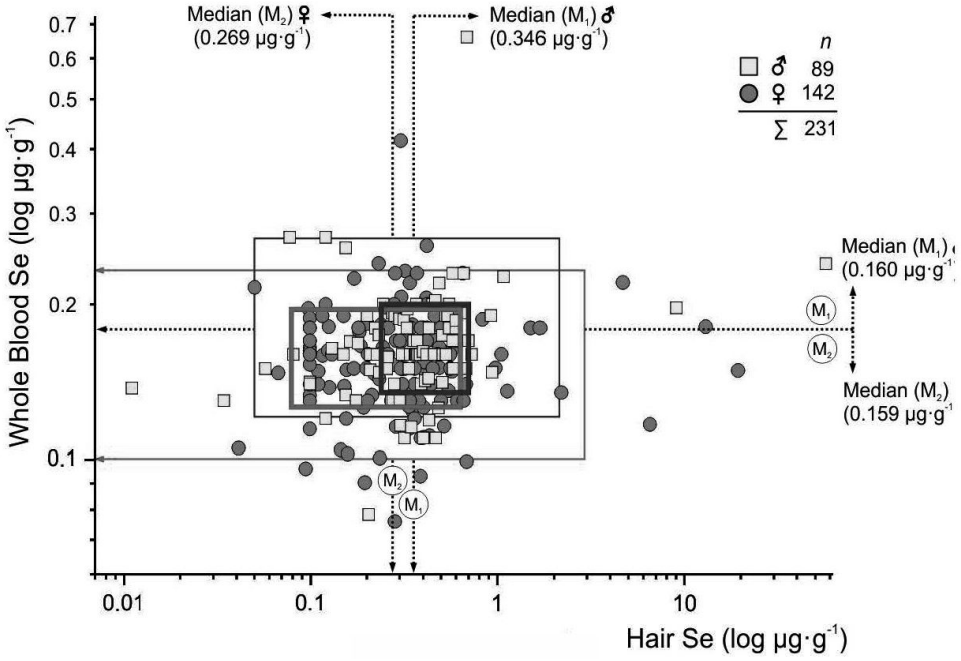


Fig. 3. *Long-term biological indicator of hair selenium and a short-term biological indicator) of whole blood selenium, are incommensurable (○ – women, ■ – men)*

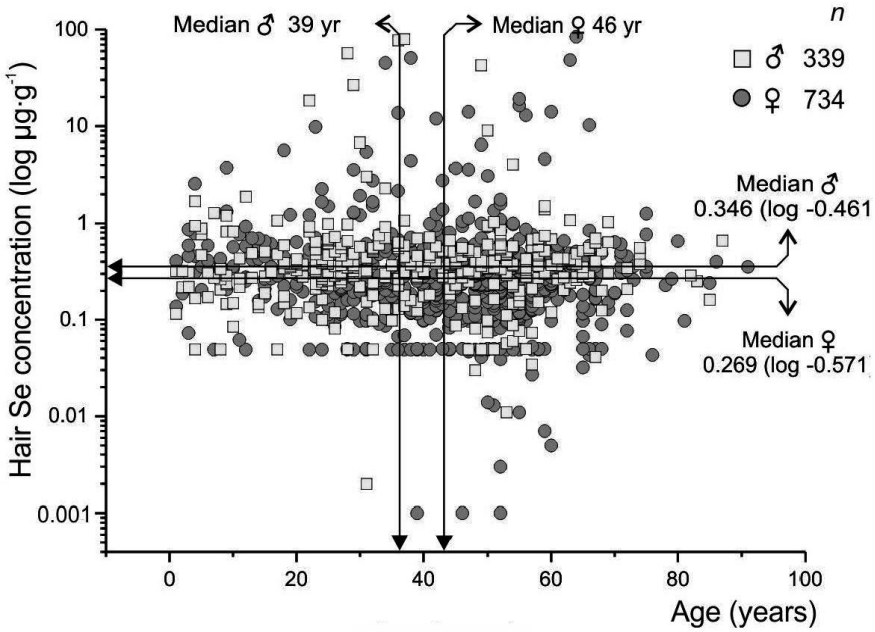


Fig. 4. *Age does not affect selenium distribution in men and women (○ – women, ■ – men)*

There was no correlation between the H·Se and WB·Se (Fig. 3), and there was no effect of age on H·Se and WB·Se accumulation (Fig. 4). Considering the median values, the observed concentrations of selenium in the hair were two times higher than those in the circulating whole blood.

The Adequate linear segment of the sigmoid bioassay curve can be further partitioned by a 60:30:10 ratio to differentiate between the respective Sparsely adequate, Adequate Optimal), and Abundantly adequate Se nutritional status. Such partition of Adequate linear segment into different subsegments may have Public Health implications (Hermann, 1971), because it allows us to closely monitor the Sparsely adequate range (60%) where selenium supplementation would be beneficiary, the Adequate (Optimal) 30% range where Se supplementation is unnecessary, and the highest 10% of Ample range where Se supplementation may induce the long term adverse health effects, respectively.

DISCUSSION

In this article we have presented a new way on how to assess the adequate selenium nutritional status by analyzing its frequency distribution in a short term biological indicator tissue of the whole blood, and long term biological indicator tissue of the hair of the Croatian population. Hair analysis was already claimed to be a reliable bio monitor tissue for detecting health problems, nutritional disease diagnosis, and environmental toxicology (Kempson, Lombi, 2011). Indeed, our preliminary results presented at TEMA 15 indicated that the Adequate Se nutritional status range ($\mu g \cdot g^{-1}$) was between 0.13–0.78 in 183 men and 0.08–0.63 in 447 women, respectively (Momčilovć et al., 2014). These results are almost identical to those of this study where the Adequate nutritional Se status have a range of 0.134–0.701 in 339 men and 0.078–0.623 in 734 women. In China they consider hair Se concentrations to be deficient when there is less than 0.250 $\mu g \cdot g^{-1}$ selenium, adequate when it is 0.250–0.500 $\mu g \cdot g^{-1}$, being highly adequate when 0.500–3.000 $\mu g \cdot g^{-1}$ (Tan, 1989). This study revealed that the selenium Sparsely adequate status is when there were 0.174–0.474 selenium in the hair, Adequate (Optimal) when it was 0.474–0.608, and Amply Adequate when 0.608–0.701). Apparently, there is a need for a more precise grading of Se nutritional status with the hair Adequate selenium range, and even the more so for more research of the Se upper safe limits and beyond in the future studies (Thomson, 2004).

Median H·Se concentrations were higher than those in the whole blood; apparently, hair follicle acts as a levy for removing circulating Se earlier (or more efficiently) in women than men. We do not see such discriminatory response in the whole blood where selenium is in permanent equilibration with the surrounding tissue. In difference, hair Se is the unidirectional process of incorporation, and hence a reliable time log of the actually available selenium over the time (Tan, 1989).

Various authors reported somewhat different Hair selenium median values. Indeed, median hair Se concentrations were ($\mu g \cdot g^{-1}$): 0.1 in Belgium (Carneiro et al., 2011), 0.34, 0.369, and 0.394 in three different regions in Tajikistan, Russia (Skalny et al., 2019), 0.37 in Palermo and 0.78 in Sardinia, two cities in Italy (Dongarra et al., 2011; 2012), 0.39 on Sicily, Italy (Tamburo et al., 2015), 0.54 in France (Goullé et al., 2005), 0.54 (Tamburo et al., 2016), and 0.60 again in Italy (Astolfi et al., 2020). Since hair samples from Palermo and Sardinia were all analyzed in the same laboratory in Italy, and hair samples from Croatia (this study) and Tajikistan, Russia, were also analyzed in the same laboratory in Moscow, it is evident that the observed differences in the hair Se content depended upon the geographical location (locality) where the people were living. Apparently, in the Europe, only Belgium (Carneiro et al., 2011) appears to be low and approaching close to the lower threshold for Se deficiency. It should be noted that the hair selenium data from different locations belong to the different points along the same sigmoid curve; thus it allowed for a quick comparison of the various international data bases, i.e., the local nutritional status of a certain bioelement of interest.

In China they consider hair Se concentrations to be deficient when less than 0.250 $\mu g \cdot g^{-1}$, adequate when 0.250–0.500 $\mu g \cdot g^{-1}$, and abundantly adequate when 0.500–3.000 $\mu g \cdot g^{-1}$ (Tan, 1989). Our study provides a more strict estimation on how the Se nutritional status is to be inferred and classified ($\mu g \cdot g^{-1}$), i.e., selenium Sparsely adequate when hair Se is 0.174–0,474, selenium Adequate (Optimal) when 0.474–0.608, and selenium Amply Adequate when 0.608–0.701.

Recently, Kohler et al (Kohler et al., 2018) reported how higher plasma selenium concentrations are associate with increased odds of prevalent Type 2 diabetes. Indeed, Yuan et al. (Yuan et al., 2021) showed that the hair Se values close to the upper threshold for average hair selenium of 0.600 μg^{-1} were associated with increased obesity, diabetes and lipid metabolism impairment. Our results suggest that the bottom part of the linear part of the sigmoid covering for 60%, would belong to the Sparsely adequate linear range. That range may provide suitable guidelines for monitoring the Se nutritional status during the dietary selenium supplementation. The middle or Adequate (Optimal) 30% of the linear sigmoid range would require no supplementation, and when hair selenium is Amply adequate, i.e., the upper 10% of the hair Se linear saturation uptake, the Se supplementation should be discouraged. It is pertinent to note here that it would be of great importance to simultaneously assess the iodine and selenium nutritional status since adding Se to a deficient iodine subjects may severely impair the thyroid function (Deuel et al., 2013). There is a need to elaborate the Se dietary supplementation, especially in the countries like China, where the question of Se deficiency became a matter of common population knowledge so that the people try to help themselves by active Se supplements. It would be advisable to control for the possibility of too much selenium supplement intake when subclinical selenosis (selenium toxicity) may be a silent threat.

The here presented results confirmed the advantage of the median derivatives bioassay for assessing selenium and the other bio element nutritional status (Momčilović et al., 2017). The median derivatives bioassay gets formed directly by the actually collected hair selenium data, and not by some arbitrary conceived mechanical grid model upon the data (Haggerty et al., 2013). The proposed grading of the sigmoid curve provides biologically reference values for the entire range from Se deficiency, over their entire adequate range interval, and up to the excessive and beyond it to the overt toxic exposure levels (Poulsen et al., 1997). The lower 30% of the overall Adequate Se nutritional status linear range, may be considered as a subclinical selenium deficiency suitable for selenium supplementation. With a caveat that every selenium supplementation is dependent upon the concomitant iodine nutritional status; the ratio of the selenium and iodine content in the hair needs to be close to one.

This study was in part presented at TEMA 15 (Kipp et al., 2015).

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Note

The corresponding author self citation references are available upon the e-mail request.

REFERENCES

Astolfi M.L., Protano C., Marconi E., Massimi L., Brunori M., Piamonti D., Migliara G., Vitali M., Canepari S. A new rapid treatment of human hair for elemental determination by inductively coupled mass spectrometry. Royal Soc. Chem. 2020; 12:1906–1918.

Bera S., De Rosa V., Rachidi W., Diamond A.M. Does a role for selenium in DNA damage repair explain apparent controversies in its use in chemoprevention? Mutagenesis. 2012. DOI:10.1093/mutage/ges064

Berry M.J., Larsen P.R. The role of selenium in thyroid hormone action. Endocrin. Rev.(1992; 13:207–219.

Browne M. Charter of rights is adopted in the UN. The New York Times. 2005. http://www.nytimes.com/learning/general/onthisday/big/0618.html. Accessed 9 January 2016.

Burges C. Valid analytical method and procedures. The Royal Society of Chemistry. Cambridge. 2000.

Cai H., Wang C., Yu W., Fan W., Wang S., Shen N., Wu P., Li X., Wang F. Selenium exposure and cancer risk: an updated meta-analysis and meta-regression. Sci. Reports. 2016. DOI:10.1038/srep19293

Carneiro M.F.H., Grotto D., Batista M.L., Rhoden C.R., Barbosa F. (Jr.). Background values for essential and toxic elements in children's nails and correlation with hair levels. Biol. Trace Elem. Res. 2011. DOI: 10,1007/s12011-011-9102-1

Deuel A., Aechambaud F., Caeon P. Selenium and the trhyroid gland. Clin, Endocrino,l 2013; 78:155–164.

Dongarra G., Lombardo M., Tamburo E., Varrica D., Cibella F., Cuttitta G. X12 Concentration and reference interval of trace elements in human hair from students living in Palermo. Environ. Toxicol. Pharmacol. 2011; 32:27–34.

Dongarra G., Varrica D., Tamburo E., Ándrea D. Trace elements in scalp jaor of children living in differing environmental contexts in Sicily (Italy). Environ. Toxicol. Pharmacol. 2012; 34:160–169.

Expert Group on Vitamins and Minerals. Safe upper levels for vitamins and minerals. cot.foodgov.uk 2003.

Finey D.J. (forwarded by Tattersfield P). Probit analysis. A statistical treatment of the sigmoid response curve. 2nd ed. Cambridge Univ Press. London. 1952.

Germelli C., Vinceti M. Scalriti E., Bazzani E., Berreti F., Vivoli G., Portolani M. Selenite inhibition of Coxsackie virus B5 replication implications on the etiology of Keshan disease. J. Trace Elem. Med. Biol. 2002; 16:41–46.

Goullé J.P., Mahieu L., Castermant J., Neveu N., Bonneau L., Lainé G., Bouige D., Lacroix C. Metal and metalloid multielementary ICP-MS validation in whole blood, plasma, urine and hair. Reference values, Forensic Science Intl. 2005; 153:39–44.

Griffin A.C. Role of selenium in the chemoprevention of cancer. Advanced Cancer Res. 1979; 29:419–442.

Haggerty L.S., Jachiet P.A., Huange W.P., Fitzpatrick D.A., Lopez P., Çonnell-Pisani D., Wilkinson M., Baptesdte E., Mc Inerney M.O. A pluralistic account of homology: Adapting the models to the data. Mol. Biol. Evol. 2013; DOI: 10.1093/molbev//mst228

Hermann E.R. Thresholds in biological systems. Arch Environ Health. 1971; 22:699–706.

Institute of Medicine. Dietary reference intakes for vitamin C, vitamin E, selemium, and carotenoids. Washington DC. The National Academy Press, 2000.

International Atomic Energy Agency (IAEA). Elemental Analysis of biological materials, IAEA – TEC. DOC 197. International Atomic Energy Agency. Vienna. 1980.

Kempson L.M., Lombi E. Hair analysis as a bio monitor for toxicology, disease and health status. Chem. Soc. Rev. 2011; 40:3915–3940. DOI: 10.1039/C1CS15021A

Kipp A.P., Strohum D., Brigelois-Flohe R., Schomburg L., Bechthold A., Leschik-Bonnet E., Heseker H. Geraman Nutrition Society (DGE). Revised reference values for selenium intake. J. Trace Element. Med. Biol. 2015; 32:195–199.

Kohler L.N., Florea A., Kelley C.P., Chow S., Hsu P., Batai K., Saboda K., Lance P., Jacobs E.T. Higher plasma selenium concentrations are associated with increased odds of prevalent Type 2 diabetes. J. Nutr. 2018; 148:1–8.

Momčilovć B., Prejac J., Višnjević V., Skalny A.A., Drmić S., Mimica N. A novel concept to assess the human iodine and selenium nutritional status by analyzing their frequency distribution properties in the hair. The 15th International Symposium on Trace Elements in Man and Animals (TEMA 15). June 22–26 2014. The B Resort. Orlando0, FL, USA. Abstract Book; 179.

Momčilović B., Prejac J., Momčilović R., Ivičić N., Veber D., Lykken G.I. On the same element isotope mass number (Pleiad) and the clusters of elements bearing the same mass numbers in the Periodic system – the "chesshyja" (fish skin) model. Trace Elem. Med. (Moscow). 2008$; 9(34):5–20.

Momčilović B., Prejac J., Skalny A.V., Mimica N. In search of decoding the syntax of the bio elements in human hair. A critical overview. J. Trace Elem. Med. Biol. 2017; 49:543–553.

Oppenheim A.N. Questionnaire – design, interviewing, and attitude measurement, Continuum. London. 2004.

Poulsen O.M., Holst E., Christiesen J.M. Calculation and application of coverage intervals for biological reference values-Technical Report. Pure and Applied Chemistry. 1997; 69:1601–1611.

Reilly C. Selenium in food and health, 2nd Ed. Springer, NY, New York. 2006

Reyman MP. Selenium in human health. Lancet. 2012; 379:1256–1268.

Roman M., Jitary P., Barbante C. Selenium biochemistry and its role for human health. Metallomics. 2013. DOI: 10.39/c3nut100185g.

Skalny A.V., Burtseva T., Salnikova E.V., Ajsuvakova O.P., Skalnaya M.G., Kirichuk A.A., Tinkova A.A. Geographic variation of environmental, food, and human hair selenium content in an industrial region of Russia. Environ. Res. 2019; 171:293–301.

Smylevich E., Dougherty R. Probabilistic Boolean network, Society for Industrial and Applied Mathematics. Philadelphia. 2002.

Tamburo E., Vaeeica D., Dongarra G. Gender as a key factor in trace metal and metalloid content of human scalp hair. Sci. Total Environ. 2016; 63:996–1002.

Tamburo E., Varrica D., Dongarra G. Coverage intervals for trace elements in human scalp hair are [geographic] site specific. Environ. Toxicol. Pharmacol. 2015; 19:70–76.

Tan J.A. Environmental selenium and health. Peoples Publishing House, Peking. China 1989. (in Chinese).

Tan J.A. Environmental selenium and health. Peoples Publishing House. Peking, China. 1989.

Thomson CD. Assessment of requirements for selenium and adequacy of selenium status: a review. Euro J. Clin. Nutr. 2004; 58:391–402.

World Health Organization, Selenium. in Trace elements in human nutrition and health. World Health Organization, Copenhagen. 1996; 105–120.

World Health Organization, Selenium. in Vitamin and mineral requirements in human nutrition. World Health Organization & Food and Agricultural Organization of the UN. Geneva. Switzerland & Roma, Italy. 2nd Ed. 2004; 194–216.

Yang G., Wang S., Zhou R., Sun S. Endemic selenium intoxication of humans in China. Am. J. Clin. Nutr. 1983; 37: 872–881.

Yuan L., Ma Z.F., Zhang M., Qin L., Yin X., Fan Y. Hair Se is a sensitive biomarker to monitor the effects of Se supplementation in elderly. Biol. Trace Element. Res. 2021. Accepted for publication.

Appendix A. ICP-MS ANALYSIS OF SELENIUM

The ICP-MS system was conditioned and calibrated via external calibration. The external calibration solutions containing 0.5, 5, 10 and 50 ppb were freshly prepared for every sample batch from the Universal Data Acquisition Standards Kit (#N9306225, PerkinElmer Inc.) diluted in DDIW acidified with 1% of HNO_3. In order to account for incomplete acidity and viscosity matching between calibration and sample matrices the online internal standardization with yttrium-89 and rhodium-103 was used via 7-port FAST valve. The internal standard solution containing 10 ppb Y and Rh was prepared from the stock yttrium and rhodium solutions (#N9300167 and #N9300144, PerkinElmer Inc.) in 8% of 1-Butanol ((#1.00988, Merck KGaA), 0.8% Triton X-100 (Sigma #T9284 Sigma-Aldrich, Co.), 0.02 % TMAH (#20932, Alfa-Aesar, Ward Hill, MA 01835 USA), and 0.02% EDTA acid (Sigma #431788 Sigma-Aldrich, Co).

Certified reference material GBW09101 – Human Hair (Shanghai Institute of Nuclear Research, Academia Sinica, China) was used for the quality control of the analytical data.

Plasma power ..... 1500 W
Plasma argon flow ..... 18 $L \cdot min^{-1}$
Aux argon flow ..... 1.6 $L \cdot min^{-1}$
Nebulizer argon flow ..... 0.98 $L \cdot min^{-1}$
Sample introduction system ESI ST PFA concentric nebulizer and ESI PFA cyclonic spray chamber (Elemental Scientific Inc., Omaha, NE 68122, USA).
Sampler and skimmer cone material ..... Platinum
Injector ..... ESI Quartz 2.0 mm I.D
Sample flow ..... 637 $\mu L \cdot min^{-1}$
Internal Standard flow ..... 84 $\mu L \cdot min^{-1}$
Dwell time and acquisition mode ..... 10–100 ms and peak hopping for all analytes
Sweeps per reading 1
Reading per replicate 10
Replicate number 3

DRC mode 0.55 mL·min-1 ammonia (294993-Aldrich Sigma-Aldrich, Co., St. Louis, MO 63103 USA) for Na, K, Ca, Ti, V, Cr, Fe optimized individually for RPa and RPq. STD mode for the rest of analytes at RPa=0 and RPq=0.25.

Appendix B. HAIR AND WHOLE BLOOD SELENIUM ANALYSIS

Hair selenium (H·Se) analysis. A strand of hair 5–7 cm long and weighting less than one gram would be cut with titanium coated scissors over the anatomically well-defined bone prominence at the back of the skull (lat. protuberantia occipitalis externa). The individual hair samples were further minced into strands less than 1 cm long prior to chemical analysis, stirred 10 min in an ethylether/acetone (3:1, w/w), rinsed three times with the deionized H_2O (18 MΩ·cm), dried at 85°C for one hour to constant weight, immersed one hour in 5% EDTA, rinsed again in the deionized H_2O, dried at 85°C for twelve hours, wet digested in HNO_3/H_2O_2 in a plastic tube, sonicated, and microwaved. The digested solutions were quantitatively transferred into 15 ml polypropylene test tubes. The liners and top were rinsed three times with the deionized water, and the rinses were transferred into the individual test tubes. These test tubes were filled up to 15 ml with deionized water and thoroughly shaken to mix. The samples were run in NexION 300 + NWR 2013 spectrometer (Perkin Elmer, USA). Graduation of the instrument was carried out with a monelement Perkin Elmer reference solution. We used certified GBW09101 Human Hair Reference Material (Shanghai Institute for Nuclear Research, Academia Sinica, Shanghai 201849, China to validate the quality of the analytical work.

Whole blood selenium (WB·Se) analysis. Whole blood was drawn by venipuncture from *cubital* vein and collected into green-cup Vacuette collecting tubes (#454082 LotA13030M7m Greineer Bio On International AG Kremsmunster, Austria) which were randomly assigned for the ICP-MS analysis. Whole blood samples of 0.5 ml were digested in a microwave oven with 0.1 ml of HNO3 at 175 ºC. Blood standards were liophylised Seronorm TM Trace Elements Whole Blood Reference Standards Level 1 (OK 0036, Level 2 (MR 9067), and Level 3 (Ok 0337) for selenium in the whole blood (SERO AS, Bilingstadt, Norway). Five ml of redistilled H_2O were added to every reference standard and stirred gently at a room temperature for two hours to equilibrate. One ml of such equilibrated standard was pipetted in 25 ml quartz glass vial, dried at 105 °C for 24 hours. The microwaved samples were dissolved in 5 ml of redistilled H_2O with 0.1 ml of H_2O_2 added.

Appendix C. Hair selenium median derivatives bioassay (Population Size, PS = 1.000)

Median (M0, µg·g^{-1})	
Median Derivative Downward (Descending) Branch (D0, PS/2 = 0.500) Descending Median Derivatives	Median Derivative Upward (Ascending) Branch (U0, PS/2 = 0.500) Ascending Median Derivatives
D1 = D0/2 0.250	U1 = U0 + U0/2 0.750
D2 = D0/4 0.125	U2 = U1 + U0/4 0.875
D3 = D0/8 0.062	U3 = U2 + U0/8 0.937
D4 = D0/16 0.030	U4 = U3 + U0/16 0.969
D5 = D0/32 0.016	U5 = U4 + U0/32 0.983
D6 = D0/64 0.008	U6 = U5 + U0/64 0.992

We studied the frequency distribution of hair selenium (H·Se) median derivatives to assess the selenium nutritional status. First, we assess the median (M0) hair selenium concentration of our subject population. By definition, one half of the studied population was above the median (upward median branch, U0), and the other half was below the median (downward median branch, D0). Hence, the population size (PS) for M0 is the sum of the respective upward and downward median branches around the central inflection "hinge" M0, i.e., PS = U0 + D0 = 0.5 + 0.5 = 1.0. Both the respective upward and downward median branches can be further divided in the same “median of median” way into a series of sequential median derivatives (U0,1,2,3,…,*n*–1, *n* and D0,1,2,3,…,*n*–1, *n*). For every median derivative of the population, the actual hair selenium concentration can be identified. Thus, instead of mechanically throwing the preconceived percentile grid upon the observed data, we inferred the median derivative grid out from the data set itself (Smylevich, Dougherty, 2002).

НОВЫЙ НЕИНВАЗИВНЫЙ СПОСОБ ОЦЕНКИ АЛИМЕНТАРНОЙ ОБЕСПЕЧЕННОСТИ СЕЛЕНОМ *IN VIVO*

Ю. Преяч[1], С. Морович[2], С. Дрмич[3], Я. Морович[4], З. Пишль[5], Б. Момчилович[6]

[1]Университетский больничный центр Загреба, отделение онкологии, Загреб, Хорватия; e-mail: juraj.prejac@gmail.com

[2] Медицинский центр Авива, Загреб, Хорватия; e-mail: Sandra.morovic@poliklinika.aviva

[3] Клиническая больница Дубрава, отделение клинической психиатрии, Загреб, Хорватия; e-mail: stipe.drmic@gmail.com

[4] Centar IGW d.o.o. Gestalt d.o.o., Загреб, Хорватия; e-mail: jadran.morovic@zg-tcom.hr

[5] Институт изучения и развития устойчивых экосистем (IRES), Велика Горица, Хорватия; e-mail: pisl@net.hr

[6] Институт изучения и развития устойчивых экосистем (IRES) Загреб, Хорватия; e-mail: berislav.momcilovic@gmail.com

РЕЗЮМЕ. Селен (Se) является важным микроэлементом в питании человека. Целью данного исследования было оценить алиментарную обеспеченность селеном путем анализа частотного распределения содержания селена в индикаторных биологических образцах – долгосрочном (волосы, H·Se) и краткосрочном (цельная кровь, WB·Se). Селен в волосах проанализирован у 1073 практически здоровых взрослых хорватов: 339 мужчин (♂) и 734 женщин (♀); селен в цельной крови проанализирован в случайной подвыборке из 91 мужчин и 143 женщин. Образцы анализировали методом ИСП-МС в Центре биотической медицины (Москва, Россия). В результате не выявлено значимых половых различий в адекватном линейном референтном диапазоне содержания Se, который составил для H·Se 0,078–0,701 мкг/г, а для WB·Se 0,120–0,200 мкг/г. Концентрация H·Se ниже 0,078 мкг/г и WB·Se ниже 0,120 мкг/г указывает на дефицит селена. Расчетные верхние адекватные пределы содержания селена для H·Se и WB·Se установлены на уровнях 0,701 и 0,200 мкг/г соответственно. Предполагается, что линейный сегмент построенной сигмовидной кривой логистического распределения H·Se отражает адекватный диапазон поступления Se. Этот сегмент адекватного потребления Se сам по себе может быть разделен процентным соотношением 60:30:10 на скудно-адекватный (♀ 0,078–0,405, ♂ 0,174–0,474), адекватный (оптимальный) (♀ 0,405–0,573, ♂ 0,474–0,608) и обильно-адекватный (♀ 0,573–0,623, ♂ 0,608–0,709) сегменты. Эти референтные данные о дозах необходимы для мониторинга поступления Se с питанием и приема селеносодержащих препаратов. Неинвазивная оценка статуса Se особенно важна при работе с такими группами населения, как беременные и кормящие женщины, а также грудные дети. Биоанализ медианных производных представляет собой новый подход в общественном здравоохранении для применения в общей медицинской практике.

КЛЮЧЕВЫЕ СЛОВА: селен, волосы, цельная кровь, алиментарная обеспеченность.