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Assessing the boron nutritional status by analyzing its cummulative frequency distribution in the hair and whole blood

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ABSTRACT

Boron is a non-essential ubiquitous trace element in the human body. The aim of this study was to assess boron nutritional status by analyzing boron frequency distribution in the long-term biological indicator tissue of hair and the short-term biological indicator of whole blood. Hair samples were analyzed in 727 apparently healthy subjects (263 ♂ and 464 ♀) and the whole blood boron was analyzed in the random subsample of them (80 ♂ and 152 ♀). Samples were analyzed by the ICP-MS at the Center for Biotic Medicine, Moscow, Russia. The adequate reference range for hair boron concentration was ($\mu g \cdot g^{-1}$) 0.771- 6.510 for men and distinctly lower 0.472–3.89 for women; there was no detectable difference in the whole blood boron for the adequate reference range between men (0.020–.078) and women (0019–0.062). Boron may play an essential role in the metabolism of the connective tissue of the biological bone matrix.

1. Introduction

Boron (B) is a non-essential trace element, but an element of many beneficial biochemical and metabolic functions for human health and well-being [1,2]. Boron has an integrative role in the areas of bone metabolism [3,4], vitamin D metabolism [5], joint health [6,7], immunity [8], mental acuity [9], wound healing [10], and proper functioning of endocrine system [4]. In many instances, boron does this by being an essential co-partner with other substances to fine-tune many human physiologic interactions [2]. These actions appear to involve at least two major biochemical mechanisms in which boron plays a vital role [11]. In combination with vit D boron has a positive effect on slowing down of the prostate cancer development [12–14]. Boron is also a key player in the boron neutron capture therapy (BNCT), a selective radiation therapy of thermal neutrons for brain glioma neoplasia, cancer of the prostate, lung cancer, and other malignancies [2,15].

The contemporary USA diet contains on average 3 mg of B per kg (range 1.0 − 5.0) [16]; blood values were reported to be 0.1 − 0.2 $\mu g \cdot g^{-1}$ [16,17], and that in the hair about 0.85 $\mu g \cdot g^{-1}$ [18–20], whereas the Acceptable Safe range for boron in the food are 1.0 − 13.0 $mg \cdot day^{-1}$, and the Upper Tolerable Level (UL) for adults is set at 20 $mg \cdot day^{-1}$ [21]. No observed adverse effect level (NOEL) is set and the lowest observable adverse effect level (LOEL) is set at 9.6 $mg \cdot d^{-1}$ and 13.3 $mg \cdot d^{-1}$, respectively [21].

The aim of this study was to assess boron nutritional status (environmental exposure), by analyzing boron frequency distribution in the long-term biological indicator tissue of hair and in the short-term

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biological indicator tissue of whole blood.

2. Subjects and methods

This prospective, observational, cross-sectional, and exploratory epidemiological 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 [22], and the complementary Croatian national bylaws and regulations. Every subject gave his/her written consent to participate in the study and filled out a short questionnaire on his/her health status and medical history (data not shown) [23]. Data on hair shampooing were also recorded to control for the possible external boron.

Hair boron (^{B}H) was analyzed in a random sample of 727 apparently healthy adults (263 Men, 464 Women). Whole blood (^{B}WB) was analyzed in a subset of 212 subjects (152 women and 80 men); the median age of women and men was 47 and 41.5 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 were fed their usual home prepared mixed mid-European diet, and none of them have reported an adverse medical health condition.

Hair boron (^{B}H) and whole blood boron (^{B}WB) were analyzed with the inductively coupled plasma mass spectrometry ICP-MS (Elan 9000, Perkin Elmer, USA) at the Center for Biotic Medicine (CBM), Moscow, Russia. The CBM is an ISO Europe certified commercial laboratory for analyzing bioelements (macro elements, trace elements, and ultratrace elements) in different biological matrices [24,25]. CBM is also a member of the exclusive External Quality Assesment of Surrey scientific group for the quality control of the trace element analysis. Hair samples were collected over the *protuberantia occipitalis externa,* an (easily identified bony bump at the back of the skull, cut in short threads, repeatedly washed and dried. Hair boron analysis was performed following the International Atomic Energy Agency recommendations [26] and other validated analytical methods and procedures [27].

2.1. Hair boron (^{B}H) analysis

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 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.

2.2. Whole blood boron (^{B}WB) analysis

Whole blood was drawn by venipuncture from *v. cubiti* and collected into green-cup Vacuette collecting tubes (#454082 LotA13030M7 m 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 HNO_3 at 175 °C. 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 boron 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 h. The microwaved samples were dissolved in 5 ml of redistilled H_2O with 0.1 ml of H_2O_2 added.

The detection limits for B in the hair and whole blood were 0.0105 and 0.00105 $\mu g \cdot g^{-1}$, respectively. All chemicals were of proanalytical grade (Khimmed Sintez, Moscow, Russia). Our detection limits ($\mu g \cdot g^{-1}$) are ^{B}Hair 0.0105 and BWB 0.00105). Current CBM allowable hair and whole blood boron ranges ($\mu g \cdot g^{-1}$) are set at 0.00–5.00 and 0.00–0.013 for men and women, respectively. Values above that range are considered to indicate excessive boron intake. Boron belongs to the pleiad of 18 elements sharing the same mass number (number of isotopes/name of the element): 2 Li, 4 Be, 6 B, 4C, 2 N. Thus, there are two lithium isotopes sharing the same mass number with 6 boron isotopes, etc. [28].

2.3. Median derivatives

The frequency distribution of boron in the hair and whole blood samples was analyzed with the median derivative method of the log transformed data after the Gaussian frequency distribution pattern was generated.

To scrutinize the hair boron and whole blood boron concentration frequency distribution, we used the median derivative model to fit the sigmoid logistic regression function (power function) for men and women separately (Appendix B) [29,30]:

$$A_2 + (A_1 - A_2)/[1 + (x/x_0)^p]$$

Where A_1 is the initial value (lower horizontal asymptote), A_2 is the final value (upper horizontal asymptote), x_0 is the center (point of inflection) is the median (M_0 detected), p is power (the parameter that affects the slope of the area about the inflection point)(Appendix B). The Qtiplot Data Analysis and Scientific Visualization programs were used for this analysis (www.qtiplot.com).

3. Results

Boron was detected in all the 727 analyzed hair samples and in all 212 whole blood samples, respectively. After the data were log transformed, the previous skewed and kurtous boron data distribution was changed into standard Gaussian (bell shaped) frequency distribution curve for both the hair (Fig. 1.Top) and whole blood (Fig. 1.Bottom).

Median derivatives (Appendix C) were used to fit the bioassay power function sigmoid curve. The data on the upward and downward arm of the median derivatives are shown separately for men (squares) and for women (circles). The bioassay sigmoid curve [32] revealed that there is a linear segment of median derivatives covering the range of ♀d2-u2 and ♂D2-U4 for ^{B}H and ♀d2–u1 and ♂D3–U1 for ^{B}WB, respectively. This linear range represents the adequate boron nutritional range where the rate of hair saturation with boron is best represented with the Power Law. Adequate hair boron concentrations of Croatian women have a linear range from 0.434-2.570 $\mu g\ g^{-1}$ (median 0.860 $\mu g\ g^{-1}$) and that for Croatian men ranged from 0.578-4.766 $\mu g\ g^{-1}$ (median1.623 $\mu g\ g^{-1}$). Indeed, the confidence intervals for the linear ranges for both ^{B}H and ^{B}WB, in both men and women, were an impressive 98–99%. The respective low linear region of the sigmoid power function curve below d2 for women and D2 for men were defined as deficient boron nutritional status regions. Similarly, the respective upper linear region of the sigmoid power function curve