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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

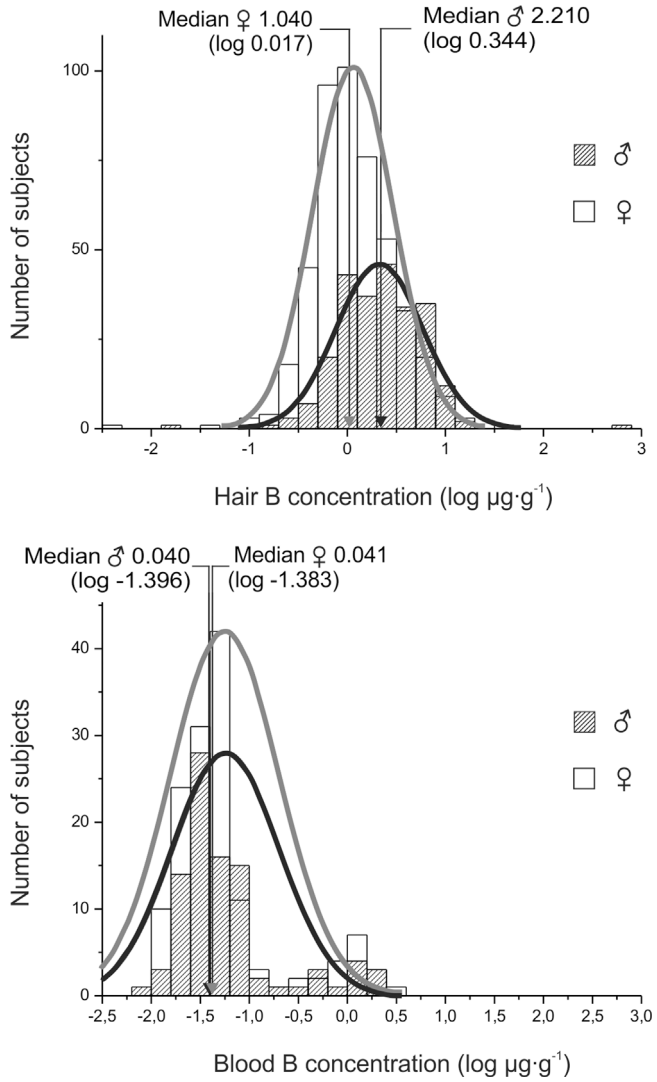


Fig. 1. Top. Hair boron frequency distribution in men (black) and women (grey) (log $\mu g \cdot g^{-1}$) Bottom. Whole blood boron frequency distribution in men (black) and women (grey) (log $\mu g \cdot g^{-1}$).

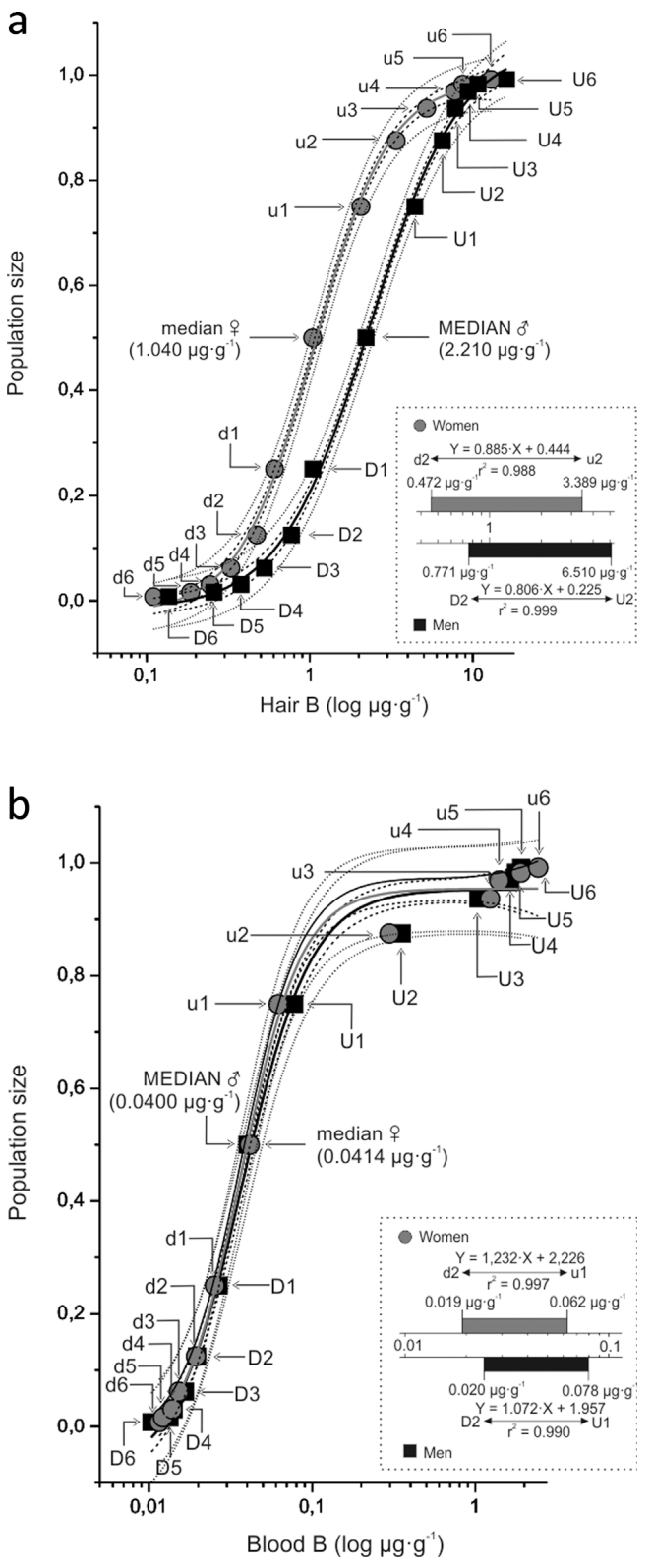
above the respective segments u2 for women (range u3–u6) and segment U4 for men (range U5–U6) were defined as an excessively high ^{B}H exposure regions. Evidently, on average, men have accumulated and retained two times more boron in their hair than women. It should be noted that this difference is evident only for the linear range segment of the observed sigmoid bioassay curves (Fig. 2).

Thus, our NOEL (non-observable effective level) for boron in the hair are boron concentrations ($\mu g\ g^{-1}$) below 0.771 for men and 0.472 for women, and our LOEL (low observable effective level values for hair boron are 6.510 for men and 3.389 for women. These values are well above the detection limits for boron and, similarly, our NOEL for the whole blood is 0.020 ♂ and 0.019♀ whereas the LOEL upper limits are 0.078 ♂ and 0.062 ♀.

There was no correlation between the ^{B}H and ^{B}WB (Fig. 3), and there was no effect of age upon the observed gender difference of ^{B}H (Fig. 4). Indeed, all the data shown in the Fig's 2, 3, and 4 demonstrated the higher accumulation of boron in the hair of men than that of women.

4. Discussion

Hair and whole blood boron concentrations in this study were comparable to the hair and whole blood boron concentrations observed



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by other authors [17–20]. Moreover, hair boron concentrations of the linear segment were higher in men than women. Indeed, the confidence intervals and prediction limits get separated at d2D2 to rejoin again at u4U6 for men and women, respectively. Thermodynamically, these results indicate the existence of a sex dependent boron multisite and cooperative binding allosteric ligand in the hair follicular cells [32].

Fig. 2. a) HaAIR. The power law bioassay sigmoid curve of hair boron median derivatives. The difference between the ^{B}H median derivatives of men n = 243 (black) and women n = 464 (grey).
D, U men downward (D) and upward (U) median derivatives; d, u women downward (d) and upward (u) median derivatives.
— Logistic function $Y = A_2 + (A_1\text{-}A_2)/(1 + (X/X_0)^p)$, – – – 0.95 confidence limit, ••• 0.95 prediction limit.
Men: $Y = 1.060 + (-0.017\text{–}1.060)/(1 + (X/2.333)^{1.581})$, $r^2 = 0.998$;
Women: $Y = 0.991 + (-0.024\text{–}0.991)/(1 + (X/1.064)^{1.890})$, $r^2 = 0.998$.
Box: Hair boron linear saturation range for ♂ and ♀ (log concentration).
See Appendix B for the median derivatives model and Appendix C for median derivatives input numerical values.
b) WbHOLE BLOOD. The power law bioassay sigmoid curve of whole blood boron median derivatives. The difference between the ^{B}WB median derivatives of men n = 92 (black) and women n = 141 (grey).
D, U men downward (D) and upward (U) median derivatives; d, u women downward (d) and upward (u) median derivatives.
— Logistic function $Y = A_2 + (A_1\text{-}A_2)/(1 + (X/X_0)^p)$, – – – 0.95 confidence limit, ••• 0.95 prediction limit.
Men: $Y = 0.954 + (-0.090\text{–}0.954)/(1 + (X/0.037)^{2.048})$, $r^2 = 0.994$;
Women: $Y = 0.955 + (-0.055\text{–}0.955)/(1 + (X/0.037)^{2.356})$, $r^2 = 0.994$.
Box: Whole blood boron linear saturation range for ♂ and ♀ (log conc).
See Appendix B for the median derivatives model and Appendix C for median derivatives input numerical values.

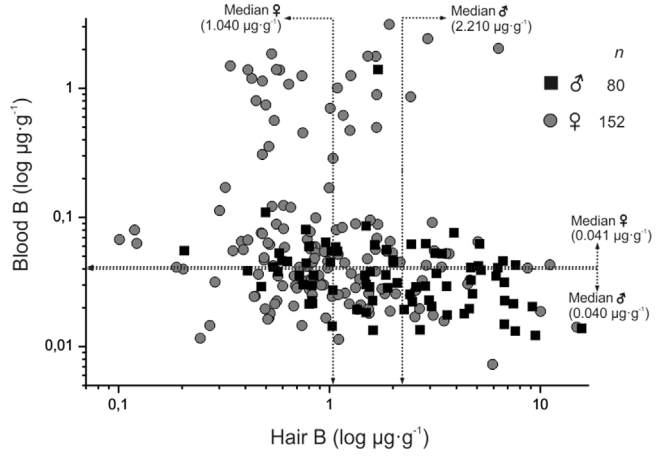

Fig. 3. Incommensurability of the hair boron (long-term biological indicator) and whole blood boron (short-term biological indicator). ● Women, ■ men.

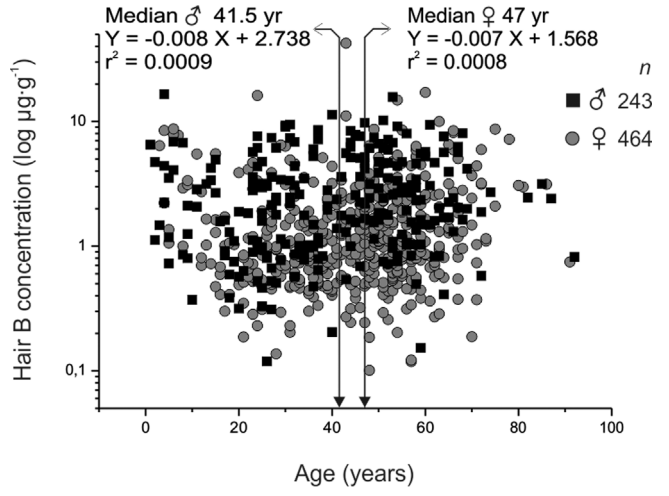

Fig. 4. Age does not affect the gender dependent difference in boron distribution in men and women. ● Women, ■ men.

However, there were no difference in confidence interval and predictive limits between the ^{B}WB linear segment for men and women.

Although boron is not considered to be an essential element its Acceptable Safe range in the food is set at 1.0–13.0 mg day^{-1}, and the Upper Tolerable Level (UL) for adults is set at 20 mg day [21]. In our parlance that would correspond with our linear reference range for women's hair boron of 0.434–2.570 µg · g^{-1} (median 0.860 µg g^{-1}) and that for Croatian men ranged from 0.578–4.766 µg · g^{-1} (median 1.623 µg g^{-1}). However, our NOEL started with the start of the liner saturation of hair with boron, i.e. above 0.424 and 0.578 for women and men respectively. Similarly, the same Institute stated the lowest observable adverse effect level (LOEL) is set at 9.6 mg d^{-1} and 13.3 mg d^{-1}, respectively [21]. That would correspond to our hair boron concentrations at u1 and U4 of 2.055 and 9.37 for women and men, respectively (Appendix C). Apparently men are more tolerable to the hair boron accumulation than women. It is pertinent to note here that the concepts of NOEL and LOEL were developed for pharmacology and toxicology and not primarily for nutrition. Indeed, in pharmacology & toxicology we have to prove if some agent (in this case a bioelement) will have or not have the desired effect.

Since boron appears to be related to the bone metabolism [2] it is important to notice that the bone-seeking elements Ca, Mg, and Sr [24,33] have shown just the reverse pattern than boron, i.e., they were higher in the hair of women than men. Thus, both groups of elements showed the sex dependent pattern of their hair accumulation and what indicates some, at the least, partial homeostatic control. In the case of boron it is interesting that the hair boron of a two month infant contained ten time more boron than the hair of her adult mother whose hair boron concentration was within the normal range study [34]. We think that the data presented here indicate that the bone-seekers Ca, Mg and Sr are primarily involved in the bio-mineralization processes and that more attention should have been paid to the role of boron in the bone connective tissue biological matrix formation. Indeed, some claims on the positive effect of boron in the rheumatic diseases [6,35] may be attributed to the positive effects of boron upon the biological matrix itself and independently of the bone bio-mineralization process.

The most recent research has shown that boron molecular biology explains its anticancer properties [36] but, however, our results on hair boron and whole blood boron frequency distribution, does not prove the possible essentiality of boron for the men. Over a century ago, Koch's rigid deterministic (mechanistic) criteria on causal inference in bacteriology [37] were adopted for assessing the criteria for trace element essentiality. It was assumed that the trace elements behave like some foreign bacteria, although they are, and in difference to bacteria, the genuine building blocks of our bodies. Later on, in the 60s, the statistician Bradford Hill proposed a set of guidelines which may help us to assess the strength of the observed causal association, but with a clear distinction that association is not a causation [38]. Thus far, to our knowledge, that shift from the rigid causal inference to a research guidelines was not used for either assessing or reassessing, of the trace element essentiality. Moreover, the multifactorial disease issues of today indicate for us to reconsider the problem of causal inference of data integration in molecular epidemiology with some modern version of the Hill's criteria [39].

Various names were associated with the bioelements which properties do not qualify them as essential by Koch's criteria but which do have some proved beneficial functional properties. Thus, we have encountered the terms like transitionally essential elements or conditionally essential elements, and since a better name could not be found or essentiality proved, we suggest the term semi-essential bioelements for elements such as boron and strontium. It has been known since the time of Paracelsus (some five hundred years ago), that any element can be toxic depending only upon the dose [40]. Here we may only add, that the dose-rate also plays a role in an bioelement toxicity [41], as evident from the sequence of poorly defined clinical and pharmacological terms of chronic-, sub-chronic-, sub-acute-, and acute-poisoning or exposure [40].

It may be also noted that the concepts of NOEL and LOEL were developed for pharmacology and toxicology [40,42] and not primarily

for nutrition. Indeed, in pharmacology/toxicology we have to prove if some agent (in this case an bioelement) will have or not have an effect, however, in nutrition we have at the least three distinguishable response levels, i.e., a deficient, adequate, and excessive nutritional status, respectively [43].

The principal advantage of the here proposed median derivative method for assessing the boron nutritional status is that it measures directly how much boron was actually absorbed by an individual. Currently available methods on assessing boron nutritional status are based on indirect, group approximations based on measuring the amount of boron in the diet, or by measuring its content in a short-term biological indicator like urine, and what all would be condensed in an expert opinion. Further studies are needed to validate the role of human hair cumulative frequency distribution bioelement analysis for assessing the human nutritional status and exposure.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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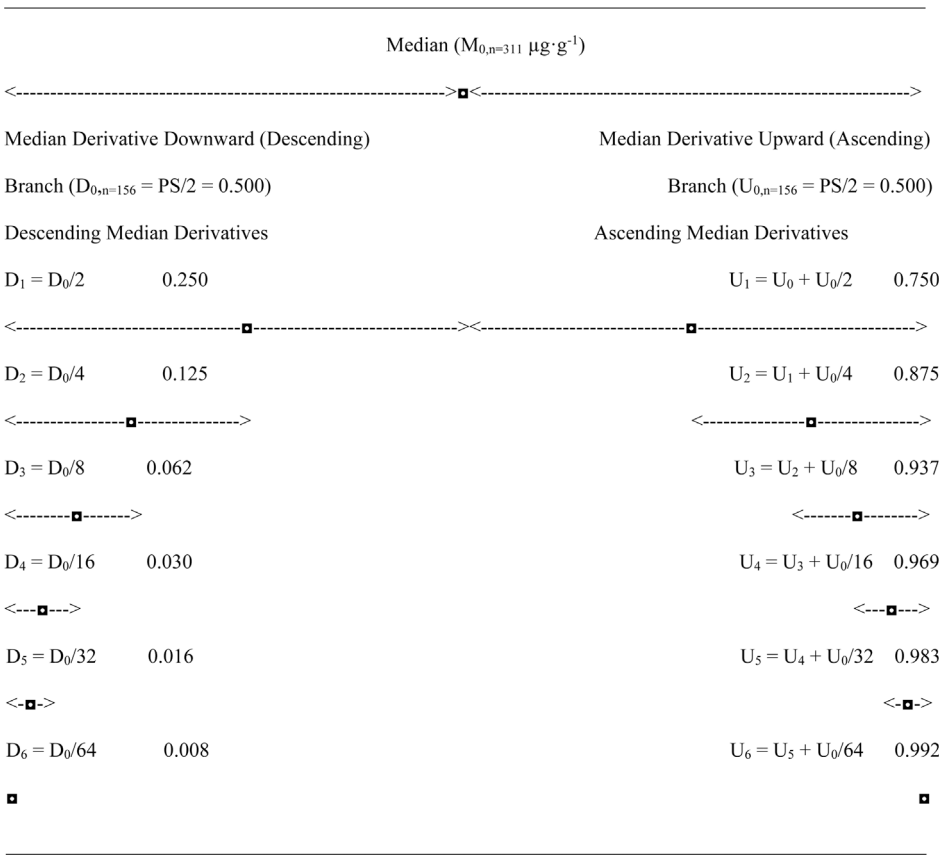
Appendix A. ICP-MS analysis of boron.

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/min
Aux argon flow	1.6 l/min
Nebulizer argon flow	0.98 l/min
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 µl/min
Internal Standard flow	84 µl/min
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 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. The hair boron median derivatives model (Population Size, PS = 1.000)



We studied the frequency distribution of hair boron (^{B}H) median and its derivatives to assess the boron nutritional status and environmental exposure. First we assess the median (M_0) hair boron concentration of our subject population. By definition, one half of the studied population was above the median (upward median branch, U_0), and the other half was below the median (downward median branch, D_0). Hence, the population size (PS) for M_0 is the sum of the respective upward and downward median branches around the central inflection "hinge" M_0, i.e., PS = U_0 + D_0 = 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 ($U_{0,1,2,3...n-1,n}$ and $D_{0,1,2,3,...n-1,n}$). For every median derivative of the population, the actual hair boron 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 [30,31].

Appendix C. Median derivatives to fit the power function sigmoid (see Appendix B). Hair and whole blood Median Derivative Concentrations (MDC).

Hair Boron											
Men (n = 243) Median (M_0) = 2.210 µ g^{-1} B						Women (n = 464) Median (M_0) = 1.040 µg g^{-1} B					
MDC	n	B	MDC	n	B	MDC	n	B	MDC	n	B
D1	122	1.044	U1	122	4.405	d1	232	0.607	u1	232	2.055
D2	61	0.771	U2	61	6.510	d2	116	0.472	u2	116	3.389
D3	31	0.526	U3	31	7.800	d3	58	0.328	u3	58	5.220
D4	16	0.378	U4	16	9.370	d4	29	0.244	u4	29	7.760
D5	8	0.258	U5	8	10.785	d5	15	0.186	u5	15	8.696
D6	4	0.136	U6	4	16.125	d6	8	0.110	u6	8	12.976

Whole Blood Boron

Men (n = 92) Median (M_0) = 0.040 μg g^{-1} B						Women (n = 141) Median (M_0) = 0.041 μg g^{-1} B					
MDC	n	B	MDC	n	B	MDC	n	B	MDC	n	B
D1	46	0.027	U1	46	0.078	d1	71	0.025	u1	71	0.062
D2	23	0.020	U2	23	0.355	d2	36	0.019	u2	36	0.298
D3	12	0.017	U3	12	1.044	d3	18	0.015	u3	18	1.228
D4	6	0.014	U4	6	1.633	d4	9	0.014	u4	9	1.401
D5	3	0.013	U5	3	1.778	d5	5	0.012	u5	5	1.897
D6	2	0.010	U6	2	1.911	d6	3	0.012	u6	3	2.426

Dd Downward arm, Uu Upward arm, Capital letters (D1–D6, U1–U6) men, small letters (d1–d6, u1–u6) women.

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