Assessing lithium nutritional status by analyzing its cumulative frequency distribution in the hair and whole blood

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Abstract. Objective: Lithium was recently proclaimed to be an essential trace element in human nutrition [1]. The aim of this study was to assess lithium nutritional status by analyzing lithium frequency distribution in the long-term biological indicator tissue of hair (H-Li) and in the short-term biological indicator tissue of whole blood (WB-Li).

Materials and methods: Hair samples were analyzed in 1,073 apparently healthy adult Croats (339 males and 734 females) and the whole-blood lithium was analyzed in a random subsample of them (91 males and 143 females). Samples were analyzed using ICP-MS at the Center for Biotic Medicine, Moscow, Russia. Results: There were no gender-dependent differences in the lithium-adequate linear reference range for H-Li and WB-Li where lithium was (µg×g–1) 0.014 – 0.100 and 3.40 – 5.65, respectively. Conclusion: Lithium concentrations below 0.014 µg×g–1 for H-Li and 3.40 µg×g –1 for WB-Li would indicate lithium deficiency. The estimated upper safety lithium limits for H-Li and WB-Li were set at 0.100 and 6.65 µg×g–1, respectively. These preliminary data are important for a long-term biomonitoring of low-level lithium supplementation.

Introduction

Lithium was recently proclaimed to be an essential trace element for human nutrition [1]. Lithium is ubiquitously present in the human diet, and daily intake of lithium from food is estimated to vary in a range from 35 µg×d–1 in Finland, to 60 – 70 µg×d–1 in the USA, and ~ 105 µg×d –1 in Turkey [2]. However, since lithium was not considered to be an essential trace element [3], there are as yet no available recommended dietary allowances or upper safety limits for this element like they exist for the other essential elements.

Lithium was shown to have a strong pharmacological potential to alleviate human depression and maniac depression when other available antidepressants have failed [4]. However, the drawback for a wider usage of lithium in the psychiatric therapy of depression is that it has to be used in high doses of 900 – 1,200 mg×d–1 [4]. Such high per os doses are close to the safety index limits of lithium, which has a very narrow therapeutic index, and could induce a plethora of toxic side effects [5]. Indeed, lithium is neurotoxic [6], it can induce nephrogenic diabetes insipidus [7], and impair thyroid gland function [8]. One of many possible factors affecting lithium toxicity is its interference with sodium metabolism [9].

Pharmacologic action of lithium in the treatment of manic-depressive psychoses is present when lithium intakes are sufficient to raise plasma lithium to 7 – 10 µg×mL–1 [2]. One of the specific peculiarities of the health effects of lithium is that suicidal rates are higher in the geographic areas with low-lithium than in the high-lithium areas [10].

Key words
lithium – nutritional status – hair – whole blood – safety limits
Moreover, the lithium therapy of depression, and in difference to other antidepressant agents, is also associated with a lower incidence of suicidality [11].

The aim of this paper is to provide evidence for assessing the lithium nutritional status by analyzing the lithium frequency distribution in the long-term biological indicator tissue of human hair and in the short-term biological indicator tissue of whole blood. We also aimed to define the provisional hair and whole-blood levels indicating lithium deficiency and to assess the lowest observed effect level (LOEL) in these two bioindicator tissues.

Materials 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 in accordance with the Declaration of Helsinki on Human Subject Research [12]. 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) [13]. Data on hair shampooing were also recorded to control for the presence of lithium (none was found).

Hair lithium (H-Li) was analyzed in a random sample of 1,073 apparently healthy adults (339 men, 734 women). Whole-blood lithium (WB-Li) was analyzed in a 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 subjects consumed their usual home-prepared mixed mid-European diet, and none of them reported an adverse medical health condition.

H-Li and WB-Li were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) (Elan 9000, Perkin Elmer, Waltham, MA, USA) at the Center for Biotic Medicine (CBM), Moscow, Russia (Supplemental material 1). The CBM is an ISO Europe-certified commercial laboratory for analyzing bioelements (electrolytes, trace elements, and ultratrace elements) in different biological matrices. CBM is also a member of the exclusive External Quality Assessment of Surrey scientific group for the quality control of trace element analysis.

Hair samples were collected over the protuberantia occipitalis externa, an easily identifiable bony bump at the back of the skull, by cutting 5- to 7-cm long strands; the longer parts of the hair strand were discarded. The collected strands were cut in short threads, repeatedly washed, and dried. H-Li analysis was performed following the recommendations of the International Atomic Energy Agency [14] and other validated analytical methods and procedures [15].

Hair lithium analysis

A strand of hair, 5 – 7 cm long and weighing less than 1 g was cut with titanium-coated scissors over the anatomically well-defined bone prominence at the back of the skull (protuberantia occipitalis externa). The individual hair samples were further minced into strands less than 1 cm long prior to chemical analysis, stirred for 10 minutes in ethylether/acetone (3 : 1, w/w), rinsed 3 times with the deionized H₂O (18 MΩ•cm), dried at 85 °C for 1 hour to constant weight, immersed for 1 hour in 5% EDTA, rinsed again in the deionized H₂O, dried at 85 °C for 12 hours, wet-digested in HNO₃/H₂O₂ 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 3 times with 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 a NexION 300 + NWR 2013 spectrometer (Perkin Elmer, Waltham, MA, USA). Graduation of the instrument was carried out with a Perkin Elmer reference solution. We used certified GBW09101 Human Hair Reference Material (Shanghai Institute for Nuclear Research, Academia Sinica, Shanghai, China) to validate the quality of the analytical work.
Whole-blood lithium analysis

Whole blood was drawn by venipuncture from the vena cubiti and collected into green-cup Vacuette collecting tubes (#454082 LotA13030M7m Greiner Bio On International, Kremsmünster, 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 °C. Blood standards were lyophilized Seronorm™ Trace Elements Whole Blood Reference Standards level 1 (OK 0036), level 2 (MR 9067), and level 3 (Ok 0337) for lithium in the whole blood (SERO AS, Billingstad, Norway). A total of 5 mL of redistilled H₂O were added to every reference standard and stirred gently at room temperature for 2 hours to equilibrate. 1 mL of this equilibrated standard was pipetted into a 25-mL quartz glass vial and dried at 105 °C for 24 hours. The microwaved samples were dissolved in 5 mL of redistilled H₂O with 0.1 mL of H₂O₂ added.

The detection limits for lithium in the hair and whole blood were 0.0105 and 0.00105 µg×g⁻¹, respectively. All chemicals were of pro analysis grade (Khimmed Sintez, Moscow, Russia). Our detection limits (µg×g⁻¹) are H-Li 0.0001 and WB-Li 0.00105. Lithium belongs to the pleiad of 14 elements sharing the same mass number (number of isotopes/name of the element): 3 He, 5 Li, 4 Be, and 2 B [16].

Median derivatives

The frequency distribution of lithium 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 H-Li and WB-Li frequency distribution, we used the median derivative model to fit the sigmoid logistic regression function (power function) for men and women separately (Supplemental material 1) A2 + (A1 – A2)/[1+(x/x0)p], where A1 is the initial value (lower horizontal asymptote), A2 is the final value (upper horizontal asymptote), x0 is the center of the median (M0 detected), p is power (the parameter that affects the slope of the area about the inflection point) (Supplemental material 2). The OriginPro 8.0 data analysis and graphing software was used for this analysis (OriginLab Corp., OriginPro Version 8.0., Northampton, MA, USA).

The bioassay sigmoid curves of log-transformed concentration data are widely used in pharmacology, toxicology, and radio-toxicology, and the semantic terminology of describing such logistic curves is well developed [17, 18, 19, 20, 21]. Indeed, (a) there is no hair bioelement deposition (response if there is no bioelement availably (lithium in this case), (b) the linear hair bioelement deposition (response) is proportional to the available bioelement concentration, and (c) at high bioelement concentrations only small further increase in...
Hair bioelement deposition can be achieved by increasing the available bioelement concentrations [18]. Hence, the terms lithium deficient, lithium adequate, and lithium excessive were chosen to describe the lithium hair and whole-blood deposition below the linear segment of the logistic sigmoid curve, deposition at the linear range part of the logistic curve, and for the part of the sigmoid curve that is above the linear part, respectively. It should be noted that even the more complex division of the logistic bioassay sigmoid curve is possible, but such divisions are beyond the scope of this manuscript.

Results

Lithium was detected in all 1,073 analyzed hair samples and in all 234 whole-blood samples. After the data were log transformed, the previous lithium data distribution (with skewed and kurtosis) was changed into standard Gaussian (bell shaped) frequency distribution curve for both the hair (Figure 1A) and whole blood (Figure 1B).

Median derivatives (Supplemental material 2, 3, Table 1) were used to fit the bioassay power function sigmoid curve. The data on the upward and downward arm of the median derivatives are shown in Figure 2 (H-Li) and Figure 3 (WB-Li) for both men and women. The bioassay sigmoid curve [17] revealed that there is a linear segment of median derivatives covering the range of d1 – u2 (for females) and D1 – U2 (for males) for H-Li and d3 – u4 (for females) and D3 – U4 (for males) for WB-Li. This linear range represents the adequate lithium nutritional range where the rate of hair and WB-Li saturation is proportional to the dietary lithium intake. Adequate hair lithium concentrations of Croatian women had a linear range from 0.014 to 0.086 µg·g⁻¹ (median 0.025 µg·g⁻¹), and that for Croatian men ranged from 0.015 to 0.100 µg·g⁻¹ (median 0.028 µg·g⁻¹). The respective low linear region of the sigmoid power function curve below d1 for women and D1 for men was defined as deficient lithium 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) was defined as an excessively high H-Li exposure. Evidently, on average, men and women have accumulated and retained the same amount of lithium in their hair.

Thus, our NOEL (no observed effect level) for lithium is composed of two parts; i.e., the lithium deficiency level and lithium adequate intake range. H-Li levels below 0.014 (µg·g⁻¹) indicate nutritional lithium deficiency in both women and men, whereas our excessive LOEL values for H-Li are those above 0.100. Similarly, our WB-Li deficiency level is below 3.450 for men and 3.243 for men.
women, whereas the excessive WB-Li levels are 5.480 for men and 5.634 for women, respectively. There was no correlation between the H-Li and WB-Li (Figure 3) values, and there was no effect of age on H-Li and WB-Li (Figure 4). However, the observed population concentrations of lithium in the hair were more than an order of magnitude lower than that in the whole blood.

Discussion

This is the first study to demonstrate what would be an adequate level of hair and whole-blood lithium in the Croatian population. Indeed, hair and whole-blood lithium concentrations in this study were comparable to the hair and whole-blood lithium concentrations observed by other authors [22, 23, 24, 25]. Hair lithium concentrations were lower than that in the whole blood and there was no gender difference in lithium content in either of the bioindicator tissues. Indeed, there was no difference in confidence interval and predictive limits within the same bioindicator, i.e., H-Li and WB-Li, respectively. Apparently, lithium absorption from the gastrointestinal tract into the whole blood is much better than the further transport within the body of lithium into the hair. It should be noted that the therapeutic doses of lithium in clinical practice are 250 – 500 µg×mL⁻¹, which is well above the observed dietary lithium intake [4].

The pharmacologic effects of lithium on depression are present when lithium intakes are sufficient to raise plasma lithium to 7 – 10 µg×mL⁻¹, and at slightly higher levels lithium becomes toxic [2]. However, in our healthy population, median lithium whole-blood levels were between 0.025 and 0.028 µg×mL⁻¹ for women and men, respectively. It should be noted that lithium concentrations are considerably lower in the plasma than in the whole blood [4]. We have estimated safety limits for whole-blood lithium at 6.5 µg×mL⁻¹ for both men and women. The mere fact that the incidence of depression is inversely related to the normally consumed dietary lithium, indicates the beneficiary health effect by much lower doses of lithium than those currently used in psychiatric therapy [10, 11]. Our recent hair multielement profile analysis has shown that the incidence of depression is strongly and positively associated with the presence of metals that make strong bonds with proteins [26, 27]. All of these metals were shown to impair the function of the Na-K-ATPase, which regulates expulsion of sodium from the cell and influx of potassium into the cell in order to maintain the electrical cell membrane potential in all the cells of our body [28]. Indeed, we have already shown how sodium and potassium concentrations were increased in the hair of depressed subjects [29], which would indicate some type of failure in the control of these two principal body cations [30].
Disturbances of metabolism of sodium and potassium have been observed in depression, and it is known that lithium can substitute for sodium in some active transport [4, 9]. Since lithium boosts the activity of Na-K-ATPase [31], we think that our results support the hypothesis that the impairment of the cell membrane Na-K-ATPase function is the major biological factor in the genesis of human depression [26, 32]. Indeed, recovery from depression was associated with an increased function of Na-K-ATPase [33].

Thus, monitoring of the hair lithium uptake may be a useful test for guiding the long-lasting low-level lithium supplementation therapy in human depression. Unlike blood, which is in permanent equilibration with the surrounding tissue, hair lithium deposition is a one-way street and thus a reliable time log of the actually absorbed lithium over time [34].

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**Conflict of interest**

The authors declare that they have no conflict of interest.

**Note**

The corresponding author will provide references he authored upon email request.

**References**


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