

Hair Iodine for Human Iodine Status Assessment

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Abstract Background: Human iodine deficiency is next to iron as the most common nutritional deficiency in developed European and underdeveloped third world countries, respectively. A current biological indicator of iodine status is urinary iodine that reflects the very recent iodine exposure, whereas some long term indicator of iodine status remains to be identified.

Objective: To explore how much iodine may be found in the human hair of an apparently healthy population, to study the frequency distribution of the observed iodine concentrations relative to the sex, and to estimate the possible risk of iodine deficiency and overexposure.

Design: We analyzed hair iodine in a prospective, observational, cross-sectional, and exploratory study involving 870 apparently healthy Croatians (270 men and 600 women). Hair iodine was analyzed with the inductively coupled plasma mass spectrometry.

Setting: Samples came from a general population from across Croatia; most of them living in Zagreb, the capital city.

Main Outcome Measures: Population (n870) hair iodine (IH) levels.

Results: The population (n870) hair iodine (IH) respective median was $0.50 \mu\text{g}\cdot\text{g}^{-1}$ (0.48 and $0.51 \mu\text{g}\cdot\text{g}^{-1}$ for men and women, respectively), suggesting no sex related difference. We studied the hair iodine uptake by analyzing the logistic sigmoid saturation curve of the median derivatives to assess iodine deficiency, adequacy and excess. We estimated the overt iodine deficiency to occur when hair iodine concentration is below 0.1 – $0.15 \mu\text{g}\cdot\text{g}^{-1}$. Then there was a saturation range interval of about 0.1 to $2.0 \mu\text{g}\cdot\text{g}^{-1}$ where the deposition of iodine in the hair was linearly increasing ($r^2=0.99$). Eventually, the sigmoid curve became saturated at about $2.0 \mu\text{g}\cdot\text{g}^{-1}$ and upward, suggesting excessive iodine exposure.

Conclusion: Hair appears to be a valuable and robust biological indicator tissue for assessing the long term iodine body status. We propose adequate iodine status to correspond with the hair iodine (IH) uptake saturation of 0.57 – $0.74 \mu\text{g}\cdot\text{g}^{-1}$ (55 – 65 %).

Background

Iodine is the heaviest essential trace element in humans.¹ Its role is critical for normal function of the thyroid gland and production of the thyroid gland hormones.² Uptake of iodide into the thyrocytes is mediated by an intrinsic membrane glycoprotein, the sodium-iodide symporter (NIS), which actively co-transport two sodium cations per each iodide anion; NIS mediated transport of iodide is driven by electrochemical sodium gradient generated by the Na⁺/K⁺-ATPase.³ Since iodine is essential for thyroid hormone synthesis, it is evident that adequate iodine intake is critical for all the metabolic processes of the human body.⁴

Humans get most of their iodine by the food intake and by iodized salt.^{5,6} Indeed, neither lack nor excess of iodine is good for human health since they both impede the normal function of the thyroid gland.⁷ Moreover, the need for iodine is not constant but depends upon the dynamic physiological status of the body like development, growth, gravidity, lactation, and physical load.⁸ Indeed, iodine deficiency may be linked either directly or indirectly to many health conditions.² The fairly common clinical entity of endemic goiter (better known as euthyroid goiter in the older German-speaking middle Europe medical writing), is the goiter induced by the iodine deficiency.⁹ Recently, we demonstrated that iodine deficiency is strongly associated with the clinical depression.¹⁰

Today's lack of iodine is one of the most common nutritional deficiencies in the world that is present in both the underdeveloped third world countries, as well as in developed European countries like United Kingdom, Italy, and Germany.^{11,12} Various methods have been suggested to assess the human iodine status and to detect the iodine deficiency and/or excess to combat nutritional iodine deficiency.¹³ Today, urinary iodine (UI) excretion is conventionally considered to be tolerable approximation of very recent dietary iodine intake. However, the determination of UI provides little useful information on the long term iodine status of an individual.¹⁴ Since even mild iodine de-

fiency should be avoided,¹⁵ there is a need for a reliable, robust diagnostic indicators for assessing the iodine body status.¹⁶

The aim of this paper is to explore how much iodine may be found in the human hair of an apparently healthy population, to study the frequency distribution of the observed iodine concentrations relative to gender, and to estimate the possible risk of iodine deficiency and overexposure.¹⁷ We have previously demonstrated the high reliability of hair for the multi-element profile analysis;¹⁸ the hair is easily accessible, easy to store and transport, and it usually has concentrations of iodine well above the detection limits necessary for accurate chemical analysis.

Methods

Ethics

This prospective, observational, cross-sectional, and exploratory study was approved by the Ethical Committee of the Institute for the Research and Development of the Sustainable Eco Systems and conducted by strict adherence to the Declaration of Helsinki on Human Subject Research,¹⁹ and to the complementary Croatian national by-laws and regulations.¹⁰ 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).²⁰

Participants and Setting

Hair iodine (I_H) was analyzed in a random sample of 870 apparently healthy adults (270 men, 600 women), 42.6 years old on average (SD 15.7, median 46), who were concerned with their health status. They came from a general population from across the country; most of them living in Zagreb, the capital city of Croatia. All the subjects were fed their usual home prepared mixed diet, and reported no adverse medical conditions. Croatia is the mid-European country with a long coastal access to the Adriatic Sea (Mediterranean) and it was reported to be the country with no apparent iodine deficiency problem; indeed, Croatia is categorized as a country having an optimal UI excretion of

100-199 $\mu\text{g/L}$.¹⁸ In Croatia dietary salt is regularly iodized, but some imported mined salt may not be iodized.²¹ Hence, we think that the hair iodine concentration of studied cohort represents the general population iodine dietary intake reasonably well.

Experimental Protocol

Hair analysis was performed by following the International Atomic Energy Agency recommendations²² and other validated analytical methods and procedures.²³ Approximately 0.5 - 1.0 g of the hair was cut from the occipital head region above the protuberantia occipitalis externa, and stored in numbered envelopes and kept refrigerated at 4°C before they were randomly assigned for analysis. Prior to chemical analysis, individual hair samples were cut less than 1 cm long, stirred 10 minutes in an ethyl ether/acetone (3:1 ww), rinsed three times with the redistilled H₂O, dried at 85°C for one hour to constant weight, immersed one hour in 5% EDTA, rinsed again in the redistilled H₂O, dried at 85°C for 12 hours, wet digested in HNO₃/H₂O₂ in a plastic tube, and sonicated. The samples were analyzed for I_H by the inductively coupled plasma mass spectrometry (ICP MS), (Elan-9000, Perkin-Elmer, USA) at the ANO Center for Biotic Medicine (CBM), Moscow, Russia; an ISO certified high tech analytical laboratory. All chemicals were pro analysis grade (Khimmed Sintez, Moscow, Russia). We used certified GBW0910b Human Hair Reference Material (Shanghai Institute of Nuclear Research, Academia Sinica, Shanghai 201849, China [CV (SD/Mean) 0.48]).²⁴

Current CBM iodine reference values ($\mu\text{g}\cdot\text{g}^{-1}$) for I_H are 0.65 - 9.00 and 0.65 - 8.00 for men and women, respectively. Our detection limit for I_H was 0.01 $\mu\text{g}\cdot\text{g}^{-1}$, and the coefficient of variation between the assays was 0.408 (SD/Mean).¹⁸ Iodine has 208 isotopes that belong to a pleiad of elements sharing the same mass number (number of isotopes/elements): 1 Ag, 7 Cd, 12 In, 21 Sn, 27 Sb, 26 Te, 24 I, 25 Xe, 17 Cs, 17 Ba, 12 La, 11 Ce, 6 Pr, and 2 Nd.²⁵

The results were expressed as a frequency

distribution, mean, and median of hair iodine concentrations. The frequency occurrence of iodine in men and women above and below the median was assessed with the Chi square test and the difference was considered to be significant when $p < 0.05$.²⁶

To further scrutinize the hair iodine concentration frequency distribution we used the median derivative model to fit the sigmoid logistic regression analysis function for men and women separately (Appendix A, page 185):^{17,27}

$A_2 + (A_1 - A_2) / [1 + (x/x_0)^p]$. Where A_1 is initial value (lower horizontal asymptote), A_2 is final value (upper horizontal asymptote), x_0 is center (point of inflection, in our case it is the median M_0), p is power (the parameter that affects the slope of the area about the inflection point). The Qtiplot Data Analysis and Scientific Visualization program was used for this analysis (www.soft.proindependent.com/gbplot.html). The same program was used to assess the exponential functions.

Results

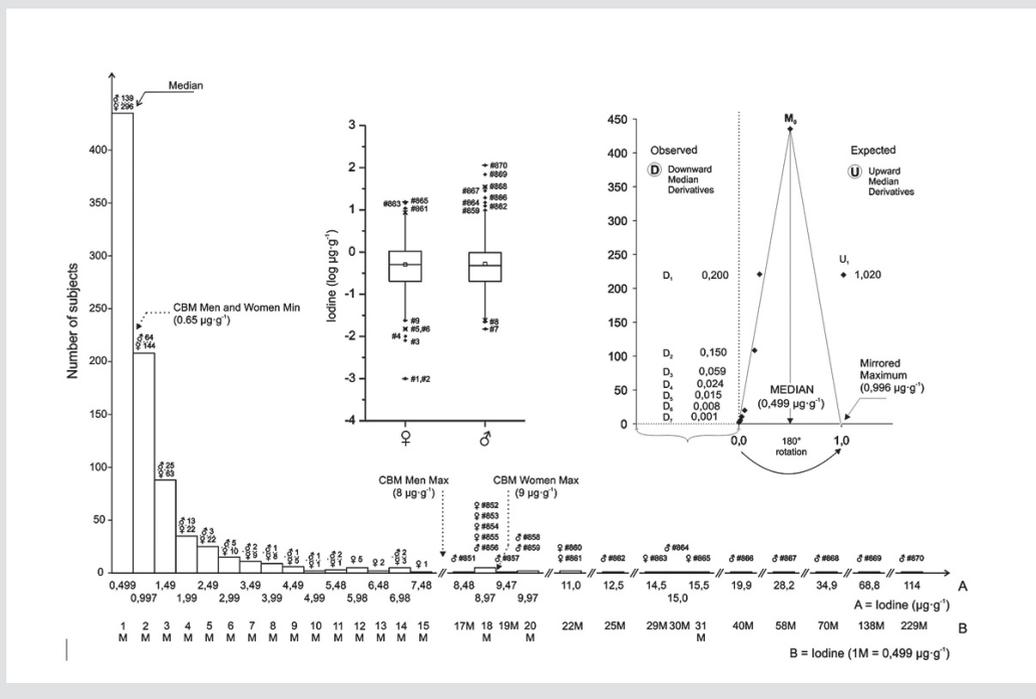
Iodine was detected in all the 870 hair samples and its concentration varied over a wide range from 0.01 to 114 $\mu\text{g}\cdot\text{g}^{-1}$, with a common median M_0 (n_{870}) = 0.50 $\mu\text{g}\cdot\text{g}^{-1}$ [men (n_{270}) = 0.48 $\mu\text{g}\cdot\text{g}^{-1}$, women (n_{600}) = 0.51 $\mu\text{g}\cdot\text{g}^{-1}$] (Figure 1, (p.178) abscissa scale A). Evidently, hair has an impressive iodine binding capacity that covers a scale of several orders of magnitude. The common median (M_0) value of iodine (0.50 $\mu\text{g}\cdot\text{g}^{-1}$) was used as basic unit of concentration on our X axis in Figure 1 (abscissa scale B). The hair iodine concentrations covering the range of 10 medians ($M_0 - M_{10}$) is best described by the exponential equation $Y = 492.8 e^{-0.54 X}$ ($r^2 = 0.96$). Extending the abscissa scale B beyond the M_{10} would progressively decrease the value of the correlation coefficient. Moreover, about 90% of all the hair iodine concentrations fall within the range of the first four medians ($M_0 - M_4$), suggesting that the upper normal limit of hair iodine is to be less than 2.0 $\mu\text{g}\cdot\text{g}^{-1}$. The box-plot data (Figure 1, left side insert) were log transformed to cor-

Figure 1. The hair iodine frequency distribution. Common median for both men σ (n=270), and women φ (n=600); $M_{0,n=870} = 0.50 \mu\text{g}\cdot\text{g}^{-1}$. # Individual subject number (subjects 1-870 are numbered sequentially depending upon the increasing iodine concentration), Abscissa A scale iodine $\mu\text{g}\cdot\text{g}^{-1}$. Exponential equation (Abscissa B scale M_0-M_{10}) $Y = 492.8 e^{-0.537X}$, $r^2 = 0.96$. ANO Center for Biotic Medicine hair iodine reference values ($\mu\text{g}\cdot\text{g}^{-1}$): φ 0.65 – 8.00, σ 0.65 – 9.00.

Right side insert: "Mirror" image model of the hair iodine median derivatives for assessing the tentative level of what would be (natural) iodine concentration today. M_0 median, D_1-D_7 men and women common downward (descending) median derivatives, U_1 men and women common upward (ascending) median derivative.

Left side insert: Box & whisker plot of the hair iodine log concentrations. – Min/Max, X 1% /99% percentile, \square Mean, \blacklozenge outliers, top "whiskers" = maximum, greatest value excluding outliers, bottom "whiskers" = minimum, least value excluding outliers, box: bottom line = lower quartile, 25% of data less than this value, box: top line = upper quartile, 25% of data greater than this value, box: middle line = median.

See Appendix A for model, and Table 1 for model input values.



rect for skewedness of the data, and there was no difference between the number of men and women above and below the common median ($p < 0.5$) when a Chi square test used. We checked the health data from the interview records and contacted 10 subjects that had the highest hair iodine; in six of them the diagnostic X-ray contrast me-

dium was used within the six month period preceding sampling, for another four subjects no data could be found to help identify the source of high iodine.

We also estimated what would be an expected normal range of hair iodine concentrations if the hair iodine follows the simple first order kinetics of deposition (Figure 1, right

Table 1. Hair iodine median derivative concentrations (MDC) for Men (D₁-D₆ downward MDC, U₁-U₆ upward MDC) and Women (d₁-d₆ downward MDC, u₁-u₆ upward MDC).

MEN Median(M0) n270 = 0.48 µg·g ⁻¹ Iodine						WOMEN Median(M0) n600 = 0,51 µg·g ⁻¹ Iodine					
MDC	n	Iodine	MDC	n	Iodine	MDC	n	Iodine	MDC	n	Iodine
D1	135	0.20	U1	135	0.96	d1	300	0.20	u1	300	1.06
D2	68	0.15	U2	68	1.77	d2	150	0.15	u2	150	1.96
D3	34	0.15	U3	34	4.32	d3	75	0.15	u3	75	3.25
D4	17	0.09	U4	17	9.60	d4	38	0.08	u4	38	5.38
D5	9	0.04	U5	9	19.50	d5	19	0.03	u5	19	6.89
D6	5	0.02	U6	5	34.90	d6	10	0.02	u6	10	8.78

Common Median (M0) n870 = 0.50 µg·g⁻¹ Iodine

side insert). Indeed, if we rotate the observed triangle [D₇·Median·M₀] by 180° around the Median·M₀ axis, we get the mirror image that would satisfy the theoretical premise of first order kinetics.¹⁷ We would suggest, according to this “mirror” based approximation, a hair iodine level of 1.0 µg·g⁻¹ the limit for adequate body iodine metabolic status.

Based upon the comparative logistic sigmoid curve of hair iodine median derivatives we suggests that iodine concentration below 0.09 µg·g⁻¹ for both men and women entails the overt iodine deficiency (Figure 2, p.180). This sigmoid curve was fitted with the data shown in Table 1 (above). Evidently, because of such low hair iodine concentrations body metabolism is in great need of iodine, so that little may be left for the hair follicle and hair growth, which may be used to explain the poor hair quality of iodine deficient persons; thyroxine advances the onset of anagen in resting hair follicles.²⁸ Above the lower horizontal asymptote hair iodine concentration, there is progressive linear upward trend of iodine accumulation in the hair that is characteristic of a physiological saturation mechanism.²⁵ This distinct saturation curve begins to plateau somewhat below 2.0 µg·g⁻¹, such that the state of hair iodine oversaturation/overexposure has been reached (here the overexposure should not be identified with the toxicity). Thus, the

linear part of iodine physiological saturation dose-response curve covers the range of 0.1 – 2.0 iodine µg·g⁻¹. The hair iodine linear saturation range is shown for both men and women combined (Figure 2, Box A) and separately for both sexes (Figure 2, Box B); apparently there was no sex dependent difference in the hair iodine between men and women.

The observed linear range segment allows us to estimate the capacity of hair to become saturated with iodine and to assess what is adequate (Figure 3, p.181). Essentially, the hair iodine rate increments (Δ) resemble a three component kinetics model of enzyme kinetics.^{29,30} The first component is composed of the hair iodine increments Δ₁-Δ₆ which rises proportionally in a constant linear fashion, the second and steeper or “faster” component was linear for Δ₇-Δ₉ increments, and the third component segment of increments Δ₁₀ -Δ₁₂ rapidly approaches the hair iodine saturation level. Thus, the IH concentrations of 0.21 – 0.50 µg·g⁻¹ (saturation capacity 20 – 50%) may be regarded as iodine sparse (not deficient but low adequate), those from 0.57 – 0.74 (saturation 55 – 65%) “Genuine” iodine adequate, and concentrations of 0.86 – 1.22 (saturation capacity 70 – 80%) may be regarded as iodine plentiful (high adequate but not excessive).

Figure 2. The difference between the hair iodine median derivatives of Men $n=270$ (\square) and Women $n=600$ (\circ) combined. D, U Men downward (D) and upward (U) median derivatives, d,u Women downward (d) and upward (u) median derivatives.

– Logistic function: $A_2 + (A_1 - A_2)/(1 + (X/X_0)^p)$,

– 0.95 confidence limit, ... 0.95 prediction limit

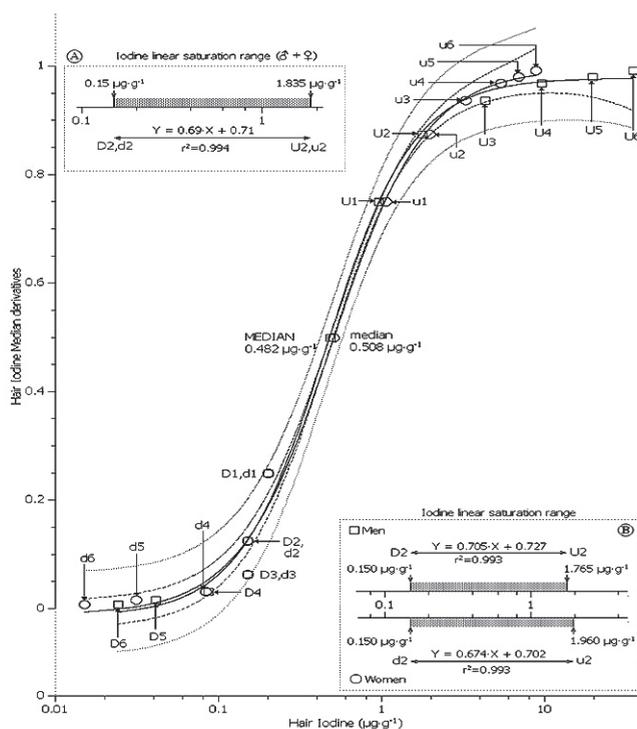
Men: $Y = 0.978 + (-0.015 - 0.978)/[1 + (X/0.456)^{1.624}]$

Women: $Y = 0.995 + (-0.011 - 0.995)/[1 + (X/0.499)^{1.532}]$

Box A: Iodine linear saturation range for ($\sigma + \varphi$)(log conc).

Box B: Iodine linear saturation range separate for σ and φ (log conc).

See Appendix for model and Table 1. for input values.

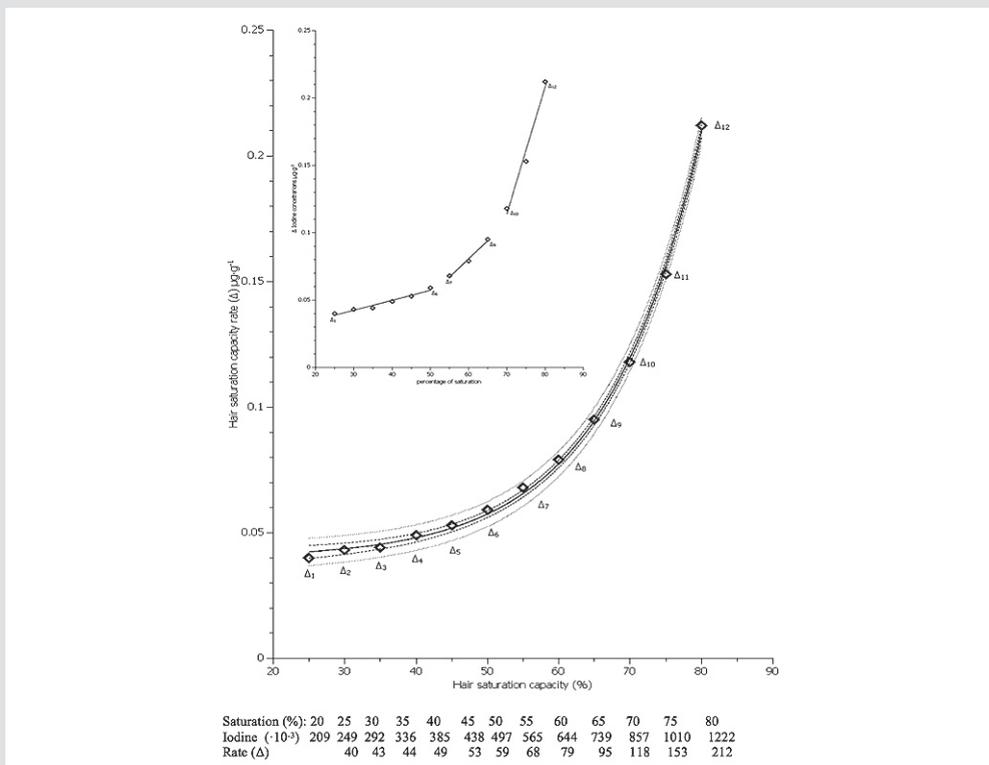


Discussion

Trace element hair analysis has been a matter of debate for years.³¹⁻³⁴ The discussion was primarily focused on problems due to external environmental contamination, shampoo and pre-analytical hair washing procedures, appropriate methods of the hair biological matrix destruction, and analytical reproducibility. Today, after a lot of refinement, the prevailing consensus is that trace

element hair analysis is a valuable method for assessing the nutritional metabolic status and assessing toxicity in a non-invasive way.^{35,36} To our surprise the observed hair iodine median was $I_{H,n=870} = 0.50 \mu\text{g}\cdot\text{g}^{-1}$ (0.48 and $0.51 \mu\text{g}\cdot\text{g}^{-1}$ for men and women, respectively), and is below the current ANO CBM concentration standard for hair iodine of 0.65-9.00 and $0.65-8.00 \mu\text{g}\cdot\text{g}^{-1}$ for men and women, respectively. That may

Figure 3. Hair Iodine linear range saturation ($I=0.15 - 1.84 \mu\text{g}\cdot\text{g}^{-1}$, $n_f + \sigma=870$)
 ----- Exponential fit $Y = 0.0397 + 0.0004e^{0.0751\cdot X}$ ($r^2=0.99$), - - - - confidence interval, prediction limit; $\Delta(\text{delta})=\text{Following}(\%) - \text{Preceding}(\%)$
 $\Delta_1 - \Delta_6 = 0.0007\cdot X + 0.2014$; ($r^2 = 0.96$)
 $\Delta_7 - \Delta_9 = 0.0027\cdot X - 0.0813$; ($r^2 = 0.99$)
 $\Delta_{10} - \Delta_{12} = 0.0094\cdot X - 0.554$; ($r^2 = 0.98$)



suggest either an inadequately high standard, or an inadequate nutritional iodine intake, or both. We think that the observed discrepancy between our current (lower) estimates of adequate iodine nutritional status and those (higher) of ANO CBM standards stems, in part, from their mechanical implementation of a preconceived percentile grid upon the untransformed (log) iodine analytical data; we prefer a model to fit the data and not the data to fit the model. Contrary to the recently published data on how Croatian population is well supplied with iodine,¹³ apparently more than half of our population has an unsatisfactory low-adequate iodine status.

Indeed, our genuine/desired adequate iodine status would require a hair iodine saturation of $0.57\text{-}0.74 \mu\text{g}\cdot\text{g}^{-1}$ and that high-adequate iodine would not exceed $2.0 \mu\text{g}\cdot\text{g}^{-1}$. What would be the hair iodine toxic level remains to be elucidated. This further emphasizes the importance of methodological challenges in the evaluation of a chosen and nutritionally influenced biomarker.³⁷

The current indicator of iodine nutritional adequacy in Croatia (urinary iodine) provided too “optimistic” results in regard to the population iodine status, and a large low level iodine population segment may be lurking beneath the cover of an inad-

equately chosen indicator, i.e., urinary iodine. Furthermore there may be a problem in supplying and/or usage of iodized salt to/by the public. We have been under constant pressure to reduce the daily salt intake for decades, and thus reduce available dietary iodine. Our method of analyzing trace element median derivatives^{17,27} offers a new way to accurately analyze samples with a large inherent variability and skewed population frequency distribution. Indeed, the logistic model of median derivatives presentation allows for direct visualization of individual trace element dose-rate phenomena; an Ostwald type of sigmoid (S) curve which can be used to describe the velocity of transformation at any instant, which is proportional to the amount of material that is undergoing the change, i.e., the growth of hair and hair iodine incorporation.³⁸

Based upon the results of this study we suggest hair iodine can be used as a valuable and robust indicator of the long term dietary iodine exposure. Growing at a pace of about 0.3–0.4 mm/day, hair is the memory tissue where the elements are irretrievably accrued; hair is the memory log of the intermediary metabolic events in homeostatic control of all the essential elements. At the same time, blood iodine and/or urinary concentrations are indicative of a short time internal balancing of this element between the various tissue compartments before it is rapidly excreted from the body. This difference in time scale for different biological indicator tissue of hair iodine vs. urinary iodine readily explains why they are incommensurable for the meaningful comparison.³⁹ Hair is itself a dynamic tissue structure – some 90% of hair follicles are active (anagen phase), some 10% are dormant (telogen phase), and some degenerate only to rise anew some other time.²⁸ Moreover, the rate of cell division in the human hair follicle is second only to the bone marrow cells, and should accurately mirror the metabolic changes within the body.⁴⁰ Hair iodine is the end point of the intermediary iodine metabolism that amalgamate all the preceding dynamic differences of intermediary metabolism and their equilibration

– starting with the iodine dietary intake and its mixing with the gastrointestinal juices, iodine bioavailability, individual differences in iodine absorption, interaction of iodine with the other elements, the presence of available vitamins, difference in the hormone status, the level of physical activity, metabolic turnover, age, and sex; to name the most prominent. Indeed, the initial diet is only a part of the gastrointestinal input into the “black box” of intermediary metabolism before its output end point is expressed in some relevant bio-indicator tissue;^{41,42} the relationship between the entry point of dietary iodine and its end point of hair concentrations does not proceed in a linear fashion given all the dynamic impacts of intermediary metabolism.

The primary goal of this paper is to draw attention of clinicians and other public health personnel that body iodine status is presented inadequately by measuring the thyroid gland hormones, instead of measuring iodine directly in some suitable biological matrix tissue like hair.¹⁰ Indeed, when iodine intake is abnormally low, adequate secretion of thyroid hormones may still be achieved by marked modification of thyroid activity. This adaptation to iodine deficiency is triggered and maintained by increased TSH stimulation.² It is pertinent to note here that low concentrations of iodine stimulate thyroid hormone synthesis independently of TSH.⁴³ We think that the newly implemented Clinical Practice Guidelines for Hypothyroidism in Adults⁴⁴ should be expanded to include hair iodine analysis. Indeed, evidence based iodine status assessment with hair iodine, would provide a reliable guide for proper iodine prophylaxis in preventing endemic goiter and would help personalized health protection in people under increased metabolic energy demands.⁸

Conclusion

We analyzed hair iodine in 870 apparently healthy subjects (270 men and 600 women) with the ICP MS. Hair appears to be a valuable and robust biological indicator tissue for assessing long term iodine body status. We propose the “genuine” adequate

iodine status to correspond with the hair iodine (IH) concentrations of 0.57–0.74 $\mu\text{g}\cdot\text{g}^{-1}$, i.e., 55–65% of hair iodine uptake saturation capacity.

Acknowledgements

This study was supported in part by the Croatian Ministry of Science, Education and Sport grant No. 292-0222412-2405 (formerly No. 022-0222412-2405). The generous philanthropic support of the RCM, Isle of Man, UK to the first author is greatly appreciated.

Competing Interests

The authors declare no competing interests.

Statement of Human Rights

This study was approved by the Ethical Committee of the Institute for the Research and Development of the Sustainable Eco Systems, Zagreb, CROATIA and the permission was granted by the Croatian Ministry of Science, Education and Sport.

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Appendix A. The median derivatives model (Population Size, PS = 1.000)

Median ($M_{0,n=870} = 0.499 \mu\text{g}\cdot\text{g}^{-1}$)	
<-----> □ <----->	
Median Derivative Downward (Descending)	Median Derivative Upward (Ascending)
Branch ($D_{0,n=435} = PS/2 = 0.500$)	Branch ($U_{0,n=435} = PS/2 = 0.500$)
Descending Median Derivatives	Ascending Median Derivatives
$D_1 = D_0/2$ 0.250	$U_1 = U_0 + U_0/2$ 0.750
<-----> □ <----->	
$D_2 = D_0/4$ 0.125	$U_2 = U_1 + U_0/4$ 0.875
<----- □ ----->	
$D_3 = D_0/8$ 0.062	$U_3 = U_2 + U_0/8$ 0.937
<----- □ ----->	
$D_4 = D_0/16$ 0.030	$U_4 = U_3 + U_0/16$ 0.969
<-- □ -->	
$D_5 = D_0/32$ 0.016	$U_5 = U_4 + U_0/32$ 0.983
< □ >	
$D_6 = D_0/64$ 0.008	$U_6 = U_5 + U_0/64$ 0.992
□	

We studied the frequency distribution of hair iodine (H-I) median and its derivatives to assess the iodine deficiency, adequacy and excess. First we assess the median (M_0) hair iodine 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 ($U_{0,1,2,3 \dots n-1, n}$ and $D_{0,1,2,3 \dots n-1, n}$). For every median derivative of the population, the actual hair iodine 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.⁴⁵