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IZiNCG TECHNICAL BRIEF

Assessing population zinc exposure with hair or nail zinc

Hair zinc is classified as a potential- and nail zinc as an emerging-biomarker of zinc exposure by the BOND Zinc Expert Panel [1]. Currently, they are not recommended as a single assessment indicator of zinc status. However, they are recommended for further development as more data are urgently needed to confirm their sensitivity and specificity to changes in zinc nutrition and to establish reference limits or true cutoffs indicative of zinc deficiency. All data must be based on standardized procedures for the collection and washing of the specimens, and accurate, precise, and sensitive analytical techniques.

Why use hair and nail zinc concentrations as indicators of zinc exposure?

Hair zinc concentrations respond positively and significantly to supplemental zinc intake in healthy adults [2], although their response to zinc depletion is uncertain [2]. In children responses of hair zinc to supplemental zinc have been inconsistent. However, associations between low hair zinc concentrations and zinc-related functional outcomes (e.g., impaired taste acuity, appetite, and linear growth) have been reported [1]. Fewer studies have examined nail zinc as a biomarker of zinc exposure even though numerous reports have confirmed the validity of nail selenium as a biomarker of selenium exposure [3]. In healthy individuals not exposed to high levels of zinc in the atmosphere, nail zinc levels are similar to those in hair [1].

Hair and nails incorporate zinc into their matrix when they are exposed to the blood supply during synthesis within the dermal papilla or the germinal layer of the nail matrix [4]. When the growing hair or nail approaches the skin surface, it undergoes keratinization and the zinc accumulated during its formation becomes sealed into the keratin protein structures and isolated from metabolic processes. Hence, the zinc content of both the hair shaft and nails reflects the quantity of zinc available in the blood supply at the time of their synthesis, not at the time

Figure 1. Advantages of using hair or nail zinc as biomarkers

1. Concentrations are higher than in serum and urine making their measurement easier
2. Specimen collection is relatively non-invasive and samples can be collected, transported, and stored at room temperature without deterioration or need for special preservatives
3. Concentrations are not subject to the rapid fluctuations seen in serum zinc produced by a recent meal, diurnal and circadian variation, or inflammation
4. Concentrations reflect exposure over a longer, retrospective time frame than serum or urine

of sampling [4,5]. Consequently, positive correlations between hair or nails and serum zinc concentrations can only be expected in settings where zinc status is unchanged such as in chronic zinc deficiency.

The normal rate of growth for hair is about 1 cm per month [1], whereas in nails the growth rate is much slower, ranging from 1.6 mm per month for toenails to 3.5 mm per month for fingernails [5]. In cases where hair or nail growth is arrested, as may occur in severe acute malnutrition and acrodermatitis enteropathica (AE) (for hair), or onychophagia (compulsive nail biting) (for fingernails), hair or nail zinc should not be used [1,5].

Technical and biological factors affecting hair or nail zinc concentrations

Hair and nails are exposed to exogenous surface contaminants so all specimens must always be washed using a recommended procedure. Biological factors affecting hair and nail zinc concentrations include age, possibly sex, season of the year, rate of growth, and for nails, onychophagia (compulsive nail biting) [1,2]. Neither cosmetic treatments or hair color affect hair zinc concentrations [1,], and chemicals introduced by nail polish can be removed by washing [5].

Collection and washing methods for hair zinc

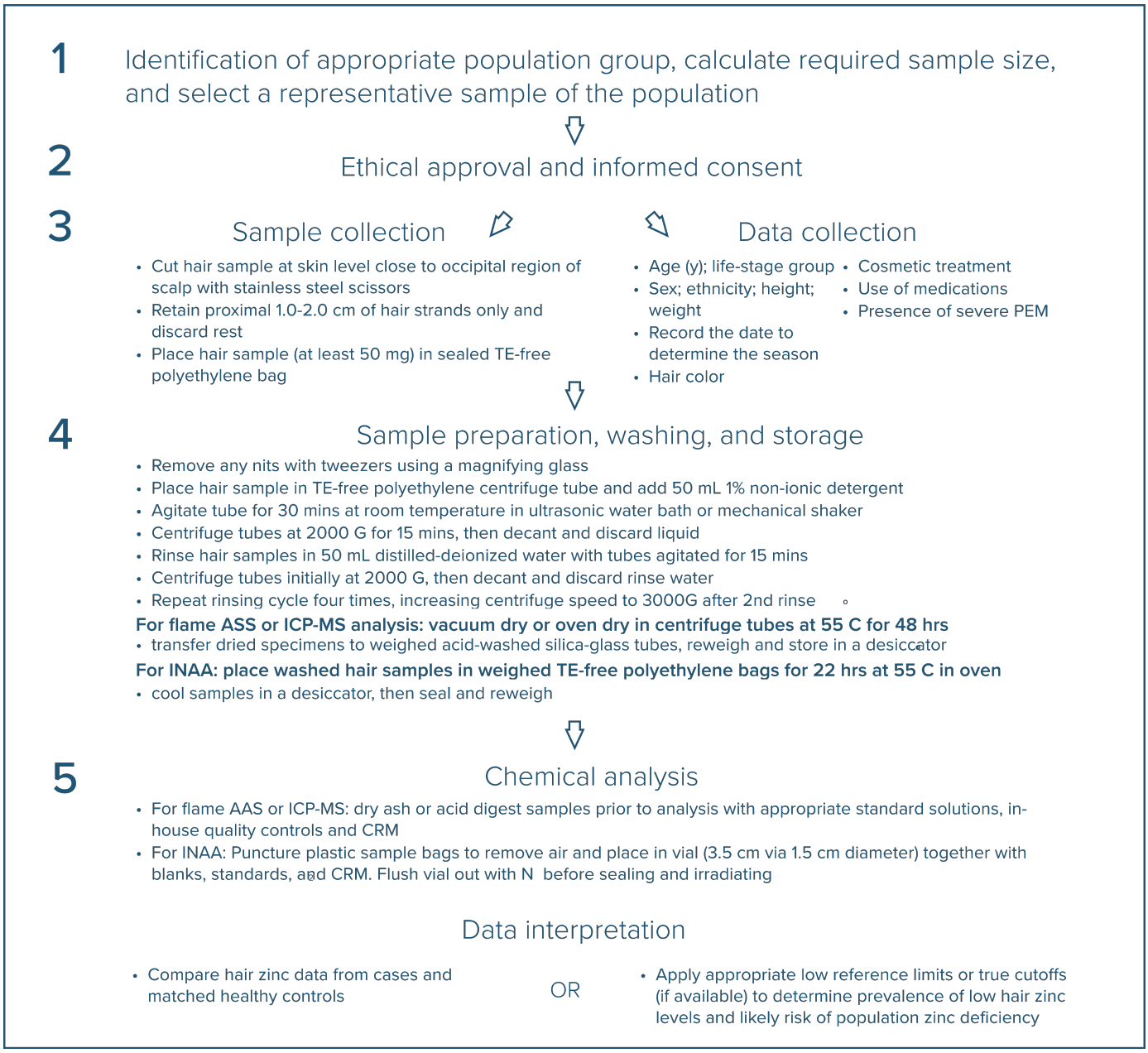
Hair specimens should be collected from a representative sample of the target population or subgroups of interest during the same season of the year. Samples (at least 50 mg) should be cut at skin level from the occipital region of the scalp (i.e., across the back of the head in a line between the top of the ears) with stainless steel scissors. Only the proximal (i.e., closest to the scalp) 1.0–2.0 cm of the hair strands should be retained. These specimens will reflect the zinc uptake by the follicles 4-8 weeks prior to sample collection provided the rate of hair growth has been normal. Hair samples should be placed in labeled trace-element (TE) free polyethylene bags for storage; any remaining hair strands should be discarded. Before washing the specimens to remove exogenous contaminants such as atmospheric pollutants, water, and sweat, any nits should be removed under a magnifying glass using Teflon-coated tweezers. For each specimen, details of the ethnicity, age, gender, hair color, height, weight, season of collection, presence of malnutrition (where relevant), and use of dandruff shampoos or cosmetic treatments should always be recorded to aid in the interpretation of the data [1].

Washing with nonionic detergents (e.g., Actinox) with or without acetone is preferred for hair as these detergents are less likely to leach bound zinc from the hair and yet are effective in removing superficial adsorbed zinc. Use of chelating agents such as EDTA should be avoided because they can cause loss of some of the tightly bound zinc that is an integral part of the hair sample [6]. After washing and rinsing, the hair samples must be vacuum or oven-dried depending on the chosen analytical method, and stored in a desiccator prior to laboratory analysis.

Collection and washing methods for nail zinc

Nail specimens should also be collected from a representative sample of the target population or subgroups of interest during the same season of the year. If possible, the nails from all fingers or toes (at least 50 mg) should be cleaned before clipping, and then stored in labeled T-E free polyethylene bags prior to washing. Several washing procedures exist, the choice depending on the chosen

Figure 2. Step-by-step guide for measuring hair zinc concentrations



method of analysis. Bank et al. [7] recommend using an aqueous nonionic detergent (e.g., Triton X-100), followed by vacuum drying for traditional analytical techniques such as flame atomic absorption spectrometry (AAS) or inductively coupled plasma mass spectrometry (ICP-MS). For non-destructive analytical methods such as instrumental neutron activation analysis (INAA) and the newer technique involving laser-induced breakdown spectroscopy (LIBS), cleaning fingernail clippings with acetone (analytical grade) in an ultrasonic bath for 10 minutes followed by drying in air for 20-30 mins is recommended [8]. All dried specimens should be stored in a desiccator after weighing, prior to analysis.

Laboratory analysis of hair and nail zinc

Several laboratory methods can be used to measure both hair and nail zinc concentrations in the washed specimens, depending on the instruments available. Traditional methods include flame AAS or ICP-MS, both of which require either ashing or chemical digestion of the washed specimens before analysis. Alternatively, tetramethylammonium hydroxide (TMAH) can be used to solubilize nails at room temperature, eliminating time-consuming

Figure 3. Step-by-step guide for measuring nail zinc concentrations

1 Identification of appropriate population group, calculate required sample size, and select a representative sample of the population (as for hair zinc).

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2 Ethical approval and informed consent (as for hair zinc)
Request participants not to trim their nails for at least 2 weeks

3 Sample collection

- Clean nails with scrubbing brush and mild detergent
- Scrape nails with stainless steel spatula
- Trim nails from each finger or toe with stainless steel scissors or nail clippers
- Place cleaned nails (at least 50 mg) in TE-free zip-lock polyethyelenebag

Data collection

- Age (y); life-stage group
- Sex; ethnicity; height; weight
- Record the date to determine the season
- Use of nail polish and color
- Use of medication on nails
- Use of foot powder

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4 Sample preparation, washing, and storage

- Place nail cuttings in a TE-free centrifuge tube with 25 mL 10% aqueous detergent (e.g., Tween 80)
- Agitate tube for 1 min at room temperature in ultrasonic water bath or mechanical shaker
- Centrifuge tube at 1000 G for 5 mins, then decant and discard supernatant
- Rinse nail samples in 25 mL distilled-deionized water with tubes agitated for 15 mins
- Centrifuge tubes as before, then decant and discard rinse water
- Repeat rinsing cycle twice
- Dry nail samples under vacuum at a pressure of 25 torr for 3 days and store in a desiccator

For INAA: place washed nail samples in weighed TE-free plastic bags for 22 hrs at 55°C in oven
Cool samples in a desiccator, then seal and reweigh

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5 Chemical analysis

- For flame AAS or ICP-MS: acid digest weighed samples using analytical grade acids

OR

- Solubilize weighed (20 mg) sample in 15 mL conical tube with 1 mL of 25% (w/v) TMAH solution, incubate at room temp overnight, then vortex 5 min and make up to volume (10 mL) with 1% ultra-pure HNO_3.

Analyse with appropriate standard solutions, in-house quality controls, and CRM

- For INAA: Weigh each dried sample in an acid-washed high-density polyethylene vial together with blanks, standards and in-house quality controls
- Flush vial out with N_2 before sealing and irradiating
- Measure decay gamma rays with high-resolution gamma-ray spectroscopy and data reduction

Data interpretation

- Apply appropriate low reference limits or true cutoffs (if available) to determine prevalence of low nail zinc levels and likely risk of population zinc

OR

- Compare nail zinc data from cases and matched healthy controls
- Investigate nail zinc for assessing pre-diagnostic status in prospective cohorts for disease risk

ashing or wet digestion, thus enhancing sample throughput [9]. Potentially, TMAH could also be used to solubilize hair, which like nails, is also composed of keratin proteins linked by disulfide chemical bonds that are split by alkaline TMAH.

Non-destructive instrumental neutron activation analysis (INAA) can also be used for both hair and nail zinc analyses. Here, the washed hair or nail specimens are placed in small, weighed TE-free polyethylene bags or tubes and oven dried for 22 hours at 55^0 C. After cooling in a desiccator, the packaged specimens are sealed and weighed, prior to irradiation in a nuclear reactor. The newer non-destructive LIBS method has been adapted to operate from a battery, and investigations for measuring fingernail zinc in situ are underway [8]. Preliminary results suggest that the in situ measurement of fingernail zinc by LIBS has potential as a non-invasive, convenient screening tool for identifying zinc deficiency in populations but may lack the precision required to generate absolute concentrations for individuals [8]. Research is ongoing to enhance the precision of the in situ method, and confirm its sensitivity and specificity to changes in zinc nutrition.

Certified reference materials (CRM)s should always be used to assess the accuracy and precision of the chosen analytical method for hair or nail zinc. Unsatisfactory quality control for hair and nail trace element analysis among laboratories is a widespread problem [10]. Depending on the assay method, in-house digested samples of a pooled hair sample or a finely cut homogenized hair should be analyzed in conjunction with a CRM to determine the precision of the analytical method [1]. Several CRMs for human hair are available (CRM 397, Community Bureau of Reference), from the Institute for Reference Materials and Measurements, Retieseweg, B-2440 Geel, Belgium, but currently no CRM exists for nail trace element analysis. Instead, in-house controls prepared from homogenous pooled samples of powdered fingernails and toenails can be prepared and spiked with several different known quantities of zinc and the recoveries measured. Alternatively, an aliquot of the in-house control can be sent to a second quality laboratory and the results compared.

Interpreting the results of hair and nail zinc concentrations

Use of reference limits indicative of risk of deficiency in population studies

There are no universally accepted reference values for hair or nail zinc concentrations [10], and as a result the use of these biomarkers for assessing risk of zinc deficiency in populations has been limited. None of the reference values published to-date have been compiled from a nationally representative reference sample of well-nourished, healthy individuals free from conditions known to affect zinc status, unlike the IZiNCG procedure used to define reference values for serum zinc [1]. In addition, standardized procedures for the sample collection (preferably throughout the year), washing, and chemical analysis of the specimens, have not been employed [10]. Hence there is an urgent need to compile a set of universal reference values for hair, fingernail, and toenail zinc concentrations. Guidelines for establishing universal reference values are available in [10].

Reference values should be represented by the geometric mean ± CV (%) or median for hair, fingernail, and toenail zinc concentrations, preferably by sex and life-stage group. Reference limits indicative of unusually low concentrations for each biomarker, represented by the 2.5^{th} percentiles for males and females for each age and life-stage

group, should also be determined. With these data, the feasibility of employing hair or nail zinc to assess the likely risk of zinc deficiency in future national nutrition surveys could be explored.

Table 1 presents the best available reference values for hair zinc (µg/g) derived from four published studies for apparently healthy children ranging in age from 3 to 15 years. Note data for both sexes are combined in three of the studies in Table 1, and the reported lower reference limits are represented by 2.5th or 10th percentile. Comparable reference values for nail zinc are not yet available. However, fingernail and toenail zinc concentrations are similar to those in hair, with mean values in adults ranging from 97 µg/g to 124 µg/g, and lower values for males than females [15]; whether age- and sex-related trends exist during childhood is uncertain.

Use of cutoffs indicative of risk of deficiency in clinical and population studies

Cutoff points, unlike reference limits, are generally based on data from individuals with either clinical or functional manifestations of a nutrient deficiency. A cutoff point has been established for hair zinc in young children but not for nail zinc. Hair zinc concentrations less than 70 µg/g (<1.07 µmol/g) have been associated with zinc-related adverse health outcomes such as impairments in linear growth, appetite, and taste acuity [1]. A higher cut-off (<110 µg/g or <1.68 µmol/g) has been used in some studies for hair samples from children collected in the Autumn/Winter months to take into account the effect of season on hair zinc concentrations [1].

Use of hair and nail zinc as biomarkers of zinc exposure in clinical studies

Additional applications for both hair and nail zinc concentrations include their use as a longer-term, retrospective measure of zinc exposure in case-control studies. Use

Table 1. Reference values for hair zinc (µg/g) based on healthy volunteers from urban areas*

Population							Reference range		Percentile	
Country	Age, y	Sex	N	Mean	SD	Median	Lower	Upper	Lower	Upper
Italy [11]	3-6	F/M	58	101	67	92	-	-	-	-
Italy [11]	6-10	F/M	96	157	50	152	-	-	-	-
Italy [11]	10-13	F/M	258	158	41	155	-	-	-	-
Italy [12]	11-13	F/M	130	189	59	179	97	330	2.5	97.5
Korea [13]	3-6	F/M	655	70	30	66	30	130		
Belgium [14]	6-10	F	218	-	-	216	150	327	10	90

N=Number of participants; SD=Standard deviation. * Modified from [10]

of nail zinc, specifically from toenails, also warrants investigation as a biomarker of pre-diagnostic zinc status in prospective cohort studies for risk of chronic diseases. For example, the association between toenail zinc and incidence of diabetes has been assessed in a prospective study (n=3,960) of US urban adults aged 20-32 y but no significant longitudinal association was found [16]. Of note, is the existence of intra-individual variability for hair and nail zinc which has the potential to attenuate estimates of association in case-control or prospective cohort studies as well as population prevalence estimates for risk of deficiency [16]. Such attenuation can be reduced by obtaining several replicate samples from each individual in the group and applying the mean value to represent true exposure.

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About IZiNCG

IZiNCG is the International Zinc Nutrition Consultative Group whose primary objectives are to promote and assist efforts to reduce global zinc deficiency through interpretation of nutrition science, dissemination of information, and provision of technical assistance to national governments and international agencies. IZiNCG focuses on identification, prevention and treatment of zinc deficiency in the most vulnerable populations of low-income countries.

IZiNCG Secretariat
UCSF Benioff Children's Hospital Oakland
Children's Hospital Oakland Research Institute
5700 Martin Luther King Jr Way
Oakland, CA, USA 94609
secretariat@izincg.org