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ORIGINAL PAPER

HAIR AS A BIOMARKER TO EVALUATE THE INTAKE OF IRON, MAGNESIUM AND ZINC IN CHILDREN*

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Abstract

Hair as a biomarker reflecting the intake of minerals could be helpful in correcting errors in the diet of children associated with inadequate consumption of magnesium, iron and zinc, and thus it can prevent their deficiency or excess in the body. The aim of the study was to evaluate the relationship between the intake of magnesium, iron and zinc and the concentration of these minerals in children's hair. The study was conducted in Warsaw (Poland) among 148 children aged 7-9. Anthropometric measurements (height, body weight) were performed on the children and the BMI values were calculated. The dietary intake of magnesium, iron and zinc in the studied children was assessed by 3-day food records method. The children were divided into four groups based on quartiles of intake of individual minerals. The concentration of iron, zinc and magnesium in the children's hair was determined by atomic absorption spectrometry (AAS). The majority of the children had a normal height (91%), weight (93%) and adjusted BMI (74%). Relationships between the intake of magnesium (r = 0.239, p = 0.007), iron (r = 0.247, p = 0.004) and zinc (r = 0.314, p < 0.001) and the concentration of these minerals in hair of the children were observed. The average intake of particular minerals in all quartiles was significantly different (p<0.001). Statistically significant differences between the quartiles of intake of magnesium, iron and zinc and the concentration of these minerals in the hair of the children were also observed but in the case of magnesium and zinc, the higher quartile of minerals intake not always corresponded to the higher concentration in the hair of these minerals. The analysis of mineral concentration in hair may be used in the evaluation of the intake of minerals by children. The sensitivity of a biomarker such as the concentration of magnesium, iron and zinc in hair depending on the intake of such minerals is larger in the case of iron, and smaller in the case of magnesium and zinc.

Keywords: magnesium, iron, zinc, minerals intake, hair, biomarker, children.

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INTRODUCTION

Children constitute a group particularly sensitive to the consequences of improper nutrition in terms of minerals, as both deficiencies as well as excesses of minerals may contribute to the deterioration of their health, disturb growth and proper development, and predispose them to the development of chronic diseases at later stages of life (Wolnicka, Taraszewska 2012). Some of the key minerals that have many important functions in the body are iron, zinc and magnesium. Magnesium and zinc are cofactors of many enzymes. Magnesium also maintains the proper electrical potential of nerve cells. Its deficiency contributes to the occurrence of disorders in many neurophysiological processes (Wang et al. 2005, Pasternak et al. 2010). Zinc deficiency causes, among others, psoriasis-like skin lesions, lack of appetite, hair loss, nervous system disorders, hypogonadism and growth inhibition in children. Both deficiencies and excesses of this mineral may contribute to the impairment of the body's immunity. In addition, excessive intake of zinc causes copper deficiency in the body, which leads to anemia (EFSA 2006, Plum et al. 2010). Iron also has a number of functions in the body. It is a component of hemoglobin and myoglobin. It takes part in the transportation and storage of oxygen and in many redox reactions. Its deficiency causes anemia, may disturb the psychomotor development of children and worsen their cognitive abilities (Wang et al. 2005, EFSA 2006, Pleskaczyńska, Dobrzańska 2011, Sahin et al. 2014). Excess of this component is also bad for health because it can cause metabolic disorders (Sahin et al. 2014).

In studies conducted in Poland, Denmark and the United States, magnesium deficiency was found in 1-49% of children, iron in 3-66% of children and zinc in 1-52% of children, depending on age and gender (Moshfegh et al. 2005, van Rossum et al. 2011, Wolnicka, Taraszewska 2012). Excessive intake of minerals was observed in 1-84% of children in the case of zinc and 1-3% in the case of magnesium and iron in studies conducted in Poland, Greece, Belgium and the United States (Eichenberger Gilmore et al. 2005, Manios et al. 2008, Pietruszka et al. 2009, Huybrechts et al. 2010, Kozyrska et al. 2010, Stewart et al. 2015).

An evaluation of nutrition and the nutritional status of children is well-founded in the prevention of deficiencies or excesses of minerals. It is particularly important to use proper biomarkers of the nutritional status which would provide reliable information about the actual nutritional status concerning a specific mineral, and at the same time would be non-invasive and easy to apply. In the case of minerals, such as iron, magnesium and zinc, it is difficult to find appropriate biomarkers of the nutritional status which would fulfill all the conditions.

The usefulness of hair as a biological material to evaluate the nutritional status of minerals and the level of toxic elements in the body has been studied for many years (Kozielec et al. 1994*a*,*b*, Karczewski 1998, Łukasiak et al. 1998, Wang et al. 2005, Dorosz et al. 2006, Dunicz-Sokołowska et al. 2006, González-Muñoz et al. 2008, Barbieri et al. 2010, Jeruszka-Bielak, Brzozowska 2011, Dongarrà et al. 2012, Sahin et al. 2014, Suliburska et al. 2015, Mehra, Thakur 2016, Llorente Ballesteros et al. 2017).

Determinations of mineral concentrations in hair are often perceived as a better biomarker of the nutritional status of minerals than determinations in blood or urine. The main component of hair is keratin, which contains amino acids (cysteine, arginine, hystidine, glutamic acid and aspartic acid) with greater affinity to metal ions than plasma albumin and plasma globulin. As a result, minerals are permanently embedded in the α-helical structure of this protein during the hair keratinization (Karczewski 1998). Mineral concentration in hair is 10-100 times greater than in blood or in urine, which facilitates an analytical procedure and increases its accuracy (Karczewski 1998, Wójciak et al. 2005, Dorosz et al. 2006, Wiechuła et al. 2007, Suliburska et al. 2015). Moreover, the mineral concentration in blood depends on factors conditioning homeostasis and may be on the physiological level even if tissue deficiencies or excesses of such minerals occur. Hair samples with a length of 2 cm from the scalp can provide information about the nutritional status in terms of minerals during the last 6-8 weeks (Karczewski 1998, Wójciak et al. 2005, Dorosz et al. 2006, Wiechuła et al. 2007, Suliburska et al. 2015). Other advantages of using hair as a biological material are non-invasive sampling, which facilitates testing in children, as well as hair durability, ease of storage and transport (Dorosz et al. 2006, Wiechuła et al. 2007, Jeruszka-Bielak, Brzozowska 2011, Mehra, Thakur 2016). Moreover, the hair of children, especially younger children, is not subjected to hairdressing procedures, which may make an examination in adults difficult. Despite many advantages of using hair as a biological material, there are still few studies assessing the relationship between the consumption of minerals and their concentration in the hair, especially in children (Karczewski 1998, Dorosz et al. 2006, González-Muñoz et al. 2008, Wojtasik et al. 2009, Jeruszka-Bielak, Brzozowska 2011, Suliburska et al. 2015). Hair as a biomarker reflecting the intake of minerals would help correct errors in the nutrition of children associated with inadequate - in relation to the demand - consumption of these nutrients, and thus prevent deficiencies or excesses in the body that may interfere with the proper development and functioning of the body. In this article, the relationship between the intake of magnesium, iron and zinc and the concentration of these minerals in children's hair was evaluated.

STUDY SUBJECTS AND METHODS

Study subjects

The study was conducted between 2014 and 2015 in Warsaw (Poland) among 148 children aged 7-9, after obtaining the consent of the Bioethical Commission at the National Institute of Food and Nutrition in Warsaw (opinion dated 19.06.2013) and consent of the children's parents.

General data concerning the children (among others: age, sex) and data concerning their health (general health, chronic diseases, food allergies) as well as the use of medications and dietary supplements containing minerals were collected using a survey designed by the research authors and completed by the parents.

The inclusion criteria involved: age (7-9), good general health, including no chronic diseases affecting the development and nutrition of children. The exclusion criteria included diseases the occurrence or treatment of which could affect nutrition (healing diets) or the nutritional status as well as the demand of the body for nutrients (kidney disease, liver disease, cardiovascular disease, cancer, diabetes, anaemia).

Evaluation of selected anthropometric parameters

In order to evaluate the general nutritional status, anthropometric measurements were performed on the children, including measurements of height and body weight. Anthropometric measurements were performed in compliance with the applicable standards. Height measurements were made using a stadiometer (Seca, type 213) ensuring the accuracy of 0.1 cm. Body weight measurements were made using electronic scales (Tech-Med, type TM-BF-001A) with the accuracy of 0.1 kg. The correctness of height and weight measurements was assessed by comparing their values to body height percentile chart adjusted to age, as well as weight percentile chart adjusted to age developed for Polish children within the OLA and OLAF projects, assuming values between the 3 and 97 percentile as the reference scope, in compliance with the interpretation provided by chart authors (Kułaga et al. 2015). Moreover, BMI (Body Mass Index) (WHO, 1988) was calculated using formula: BMI = weight (kg)/height (m)^2. BMI values were compared with BMI percentile chart adjusted to age and the results were read in compliance with the interpretation provided by percentile chart authors (underweight, normal body weight, overweight, obesity) (Kułaga et al. 2015).

Evaluation of iron, magnesium and zinc intake

In order to evaluate the usefulness of hair as a biomarker of the nutritional status in children, relationships between iron, magnesium and zinc intake and the concentration of these minerals in hair were studied.

The dietary intake of magnesium, iron and zinc in the studied children was assessed by 3-day food records method. The parents were asked to register food (products, meals, drinks) consumed by children. Head teachers helped register the food consumed at schools. Magnesium, iron and zinc intake with daily food consumption (taking dietary supplements containing iron, magnesium or zinc into account) was calculated using computer software DIETA 5.0. Subsequently, the children were divided into four groups based on intake quartiles of individual minerals (Q1, Q2, Q3, Q4). The demand for minerals in girls and boys aged 7-9 years is within the same reference range, so the group of the studied children was not divided according to sex (Jarosz et al., 2017).

Evaluation of the concentration of selected minerals in hair

Hair samples were taken from 6 places in the occipital part of the children's heads. For analysis, sections of 3 cm long hair cut off at the skin were used. The samples were stored in paper envelopes at room temperature.

The study material was prepared using the method developed by the International Atomic Energy Agency (IAEA, 1977). Hair samples were rinsed with acetone (Merck 1.00014.1000, Germany), three times with redistilled water and again with acetone. Dried hair samples were mineralized in 65% nitric acid (Merck 1.00456.1000, Germany) in a microwave oven (Mars 5, CEM, USA). The concentrations of iron, zinc and magnesium in the children's hair were determined by atomic absorption spectrometry (AAS) using a UNICAM 989 spectrophotometer by SOLAAR (Great Britain). Magnesium determination was carried out after the addition of 5% lanthanum chloride to the mineralizate. Proper analyses were preceded by the determination of iron, zinc and magnesium in the hair reference material (IAEA-085). The recovery for the hair was 96% for magnesium, 103% for iron and 97% for zinc. The coefficient of variation was 3% for magnesium, 4% for iron and 6% for zinc.

Statistical analysis of results

The results of the study were subject to a statistical analysis using IBM SPSS Statistics 24, PS IMAGO 5.0 by Predictive Solutions. The regularity of quantitative data distribution was checked using the Shapiro-Wilk test. To obtain regularity of the distribution, the data were subjected to logarithmic transformation. The significance of differences between quartiles was verified through a one-way analysis of variance (ANOVA) and *post hoc* test: LSD. Relationships between the minerals' intake and their concentration in hair were examined using the Pearson's linear correlation (parametric data). For all analyses carried out, the level of significance was $\alpha = 0.05$. The minimum number of subjects for 90% power of study was determined based on the mean concentration of magnesium in the hair of children using literature data (Kozielec et al. 1994*a*), in accordance with the guidelines of Rosner (2011). A minimum sample of 31 individuals was estimated as sufficient.

RESULTS AND DISCUSSION

In the studied group of children, girls constituted 53% and boys made up 47%. The majority of the studied children had normal (between 3 and 97 percentile) height (91%), weight (93%) and BMI (74%) in relation to their age (Table 1). According to BMI values, underweight children constituted 16%,

Table 1

Anthropometric parameters of children

Anthropometric parameter	$x \pm SD$	Min-max	Percentile*	n = 148	(%)
Height (cm)	126.3 ± 8.2	103.7 – 146.9	<3	5	3
			≥3-25	31	21
			>25-75	65	44
			>75-97	38	26
			>97	9	6
Weight (kg)	25.9 ± 6.0	13.9 – 50.8	<3	1	1
			≥3-25	30	20
			>25-75	86	58
			>75-97	22	15
			>97	9	6
BMI (kg m^{-2})	16.1 ± 2.3	12.9 – 24.2	underweight	24	16
			normal weight	110	74
			overweight/obesity	14	10

x – mean, SD – standard deviation, min – minimum, max – maximum, * according to percentile charts by Kułaga et al. (2015)

while overweight ones represented 10% of the studied group. The percentile distribution of anthropometric features obtained in the study was similar to the results achieved in studies carried out on children by other Polish authors (Kułaga et al. 2009, 2010, 2011, Malczyk et al. 2015, Kołpa, Jankowicz-Szymańska 2017).

Positive relationships between the intake of magnesium (r = 0.239, p = 0.007), iron (r = 0.247, p = 0.004) and zinc (r = 0.314, p < 0.001) and the concentration of these minerals in the hair of the studied children were observed. For the purpose of a more accurate analysis of such relationships, the studied children were divided into four groups based on the values of quartiles of the intake of particular minerals, after which the average concentration of the studied minerals between such quartiles was compared (Table 2). The average consumption of particular minerals in all quartiles was different and the differences were statistically significant (p < 0.001). Then, the concentration of the studied minerals in the hair of the children

Table 2

Dietary intake and hair concentration of magnesium, iron and zinc in children

Mineral	Dietary intake (mg)						P*
	total n = 148		Q1	Q2	Q3	Q4	
	$x \pm$ SD	range					
Magnesium	249 ± 68	135-616	201	237	291	616	<0.001
Iron	10.1 ± 3.2	5.5-22.9	7.8	9.6	11.6	22.9	<0.001
Zinc	9.6 ± 2.6	5.3-17.3	7.5	9.1	11.7	17.3	<0.001
Concentration in the hair (μg g^{-1} dry weight)							
Mineral	$x \pm$ SD	range	$x \pm$ SD	$x \pm$ SD	$x \pm$ SD	$x \pm$ SD	P*
Magnesium	17,7 ± 8.6	2.3-64.2	13.6 ± 5.2^{a}	$18.3 \pm 7.5^{b,c}$	17.4 ± 5.4^{b}	$21.4 \pm 12.2^{b,c}$	0.001
Iron	21.6 ± 10.3	9.9-74.0	13.1 ± 1.8^{a}	16.0 ± 2.2^{b}	22.0 ± 4.0^{c}	34.3 ± 11.2^{d}	<0.001
Zinc	182 ± 63.6	33.5-440.3	133.0 ± 56.6^{a}	189.8 ± 61.8^{b}	187.4 ± 46.3^{b}	217.8 ± 59.3^{c}	<0.001

* ANOVA test (after logarithmic transformation of data), $p \leq 0.05$ – statistical significance, Q – quartile of mineral intake, x – mean, SD – standard deviation, ^{a,b,c,d} values marked with different letters mean that the groups differ significantly statistically from each other (LSD test, a = 0.05)

was verified depending on the quartile of minerals' intake (Table 2). Statistically significant differences between the consumption quartiles of magnesium, iron and zinc and the concentration of these minerals in the hair of the studied children were also observed. In the case of iron, the higher the quartile, the greater its concentration in the hair of the studied children. The concentration of magnesium in the hair of the studied children was the lowest in the case of the first consumption quartile, but the concentration of the mineral corresponding to the second quartile was not statistically significantly different in comparison to the third and fourth quartile. The concentration of magnesium in the hair of the studied children in the fourth quartile of intake was different by 57% from the first quartile and by 23% from the third quartile, although it was not different from the second quartile. The concentration of zinc in the hair of children was similar taking into account the second and third consumption quartile, while it differed from the others in the case of the first and fourth quartile.

Relationships between the consumption of minerals and their concentration in hair were also observed in studies conducted by other authors. One of the earliest studies in Poland was conducted by Karczewski (1998), who compoleted his study between 1982 and 1993 with an aim of evaluating the usefulness of hair as the basic biological materials in screening tests evaluating magnesium management in the body. His study consisted of several stages. One of them involved the evaluation of the concentration of magnesium in the hair of 700 children aged 12-15 depending on their nutrition, including the amount of magnesium in their daily food intake as well as the place of residence and social conditions. The author observed that nutrition and the amount of magnesium in the daily food intake significantly affected the concentration of magnesium in children's hair and, based on this finding, he claimed that determination of magnesium concentration in hair may be used as a sensitive marker to evaluate nutrition. A relationship between the consumption of magnesium and its concentration in children's hair was also observed by Wojtasik et al. (2009). In children with celiac disease, but with normal small intestinal mucosa, who were treated with a gluten-free diet and consumed magnesium-containing dietary supplements, a higher concentration of magnesium in their hair was observed in comparison to children who were not administered any supplements. Similar results were obtained by Dorosz et al. (2006). In their study, a significantly higher concentration of magnesium in the hair of adults with a greater consumption of magnesium related to long-term supplementation of magnesium was observed in comparison to the control group of people who did not apply supplementation. In the study conducted by Jeruszka-Bielak, Brzozowska (2011), relationships between the consumption of selected groups of products (milk and dairy, meat) and vitamin D and the concentration of calcium in the hair of women aged 19-26 were observed. In the literature there are also reports on investigations indicating relationships between mineral concentration in the hair and other factors related to the consumption of minerals. In the study conducted by Sahin et al. (2014), a significantly lower concentration of iron in the hair of people with iron deficiency anemia was observed in comparison to the control group; also, positive correlations between the concentration of iron in the hair and biomarkers of the nutritional status concerning iron determined in blood were demonstrated. In the study conducted by Song et al. (2007), a positive relationship between the concentration of magnesium in hair and the bone mineral density in premenopausal women was observed.

Suliburska et al. (2015), who conducted a study among women aged 18-70 (n = 106), found no significant correlations between the consumption of calcium, magnesium, iron, zinc and copper in women and the concentration of these minerals in hair. The lack of a relationship between the consumption of minerals and their concentration in hair was also confirmed by González-Muñoz et al. (2008), who carried out tests among young adults in Spain. In that research, mineral concentration in hair could have been affected by other factors. In the study conducted by Suliburska et al. (2015), chronic diseases were observed in a significant percentage of the studied women, particularly in older age groups. Numerous women took medications, including contraceptives, regularly. Moreover, the mineral concentrations in their hair could have been affected by hair care products and hairdressing treatments, which were not considered in the studies. However, mineral concentrations in the hair of healthy children do not depend on such factors.

The existence of a relationship between the magnesium, iron and zinc intake and their content in the hair indicates the possibility of using hair as a biomarker for estimation of the adequate intake of these minerals in children. However, according to the literature data, the content of minerals in hair depends on many other factors, such as age, sex, nationality, race, hair color, using hair care products and hairdressing treatments, health and nutritional status, geographical location, environmental pollution, as well as the presence of anti-nutritive substances in a diet and the chemical form of consumed minerals (Karczewski 1998, Wang et al. 2005, Dorosz et al. 2006, Dunicz-Sokołowska et al. 2006, Barbieri et al. 2011, Jeruszka-Bielak, Brzozowska 2011, Dongarrà et al. 2012, Sahin et al 2014, Suliburska et al. 2015, Mehra, Thakur 2016, Llorente Ballesteros et al. 2017). So many factors affecting the content of minerals in hair may cause difficulties in the proper interpretation of results. Another limitation is the lack of reference values suitable for different minerals in different population groups (Jeruszka-Bielak, Brzozowska 2011). Moreover, mechanisms of the incorporation of minerals into the hair structure are not fully understood (Karczewski 1998).

CONCLUSIONS

1. An analysis of mineral concentrations in hair may be used in the evaluation of the consumption of minerals by children.

2. The sensitivity of a biomarker such as the concentrations of magnesium, iron and zinc in hair depending on the consumption of such minerals is larger in the case of iron, and smaller in the case of magnesium and zinc.

3. Due to a large number of factors affecting mineral concentrations in hair, the evaluation of the nutritional status considering iron, magnesium and zinc should be accompanied by an evaluation of nutrition, general health or use of other biomarkers of these minerals.

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