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ORIGINAL PAPER

THE DAILY INTAKE OF MINERALS AND THE HAIR CONCENTRATION OF MINERALS IN ELDERLY WOMEN*

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Abstract

The objective of study was to evaluate the daily intake of Ca, Mg, Zn, Cu, Fe, and compare these minerals in terms of their concentration in the scalp hair of 90 elderly women. The mineral intake was calculated as the difference between the food rations served and the uneaten food residue. The women consumed similar amounts of Ca, Mg, Zn, Cu, and Fe. In women over 85 years of age, there was an explicitly proportional, statistically significant relationship between the BMI and Ca content in hair, while in women aged 75-84, such a relationship was between the BMI and the level of copper in hair. A higher copper intake in the DFR significantly influenced lower levels of magnesium in the hair of the oldest women, while in the group of youngest women there was a significant, inversely proportional relationship between the calcium intake from the DFR and the zinc content in hair. A similar relationship was observed in this group between the consumption of zinc in the DFR and the calcium content in hair. The mean Ca/Mg, Zn/Cu, Zn/Fe, and Fe/Cu ratios in the hair of all subjects were as follows: 19.37, 19.70, 16.87 and 1.63, respectively. There were no statistically significant differences between aged-divided groups of women in relation to Ca/Mg and Zn/Cu ratios. The Zn/Fe ratio in the subjects' hair was significantly higher in women over 85 years old than in younger women (23.63 vs. ca 14.50), whereas the Fe/Cu ratio was significantly lower in this group than in the others (0.97 vs. ca 1.80). Significant correlations were found between age and hair-Zn and Fe concentrations. The BMI of the women correlated positively with the hair-Ca level and hair-Cu level. The Ca intake by women correlated with the hair-Zn level, as well as the Zn intake with the concentration of Ca in the hair of the analysed population. The results of the current study

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demonstrate that increased age is associated with a significant change in hair trace element content in elderly women.

Keywords: hair analysis, minerals, nutritional status, trace elements, Ca, Mg, Cu, Fe, Zn.

INTRODUCTION

In recent years, there has been growing scientific interest in elderly people and their quality of life. This is largely due to the growing number of elderly people in the western population. As life expectancy has steadily increased over time, the biological processes of aging have become ever more intensified to present a wide array of systemic diseases that affect today's senior citizens (aged 60+ years) (Gonzales-Munoz et al. 2010, Mahdavi--Roshan et al. 2015, Skalny et al. 2018).

Gerontological studies are mostly focused on broad aspects of the quality of life of elderly people, such as identifying aging-associated diseases and changes which those diseases have generated as well as finding relevant treatments and preventive measures (Ignasiak et al. 2016, Park et al. 2017). A large number of studies focus on many complex relationships among a variety of internal and external factors (Alpert et al. 2017, Wójciak et al. 2019, Byhoff et al. 2019, Gerards et al. 2021). Nutrition, lifestyle and physical activity are most frequently cited as factors in aging-associated diseases, such as osteoporosis, obesity and undernourishment, which play the most important role in determining the health status of elderly people. On the other hand, many authors look at the world of older people through the prism of their diseases. There are few data assessing the normal course of human aging, nor seeking indicators in such assessment or parameters which change over the years and to the extent of changes (Shlisky et al. 2017).

The challenge is to answer the following questions: how does the composition of the body naturally change with age and how can we stop the excessive loss of essential elements in the body? Although most of these changes in the field of body macro-composition (body weight, protein concentration, bone structure, etc.) are known, few papers mention changes in the microcomponents of the body and their loss with age. Undoubtedly, deficiencies of micronutrients in the body may be responsible for a number of processes accelerating natural aging or causing age-related diseases. Minerals are the compounds largely responsible for the body's metabolic processes, from micro-scale ones to the formation of the skeletal system or other tissues (Cisek-Woźniak et al. 2019). Assessment of minerals in the body is usually based on an analysis of their concentration in body tissues, like blood, serum or urine. Multisystem problems in the elderly make such analyses difficult or sometimes impossible. Blood collection requires invasive needle insertion into the patient's vein. In the elderly, vein fragility often occurs, which commonly prevents the use of blood as an indicator tissue. On the other hand, using blood to assess nutritional status has many drawbacks. In addition to the invasiveness of this method, homeostasis means that deficiencies of some minerals often remain invisible for a long time. Another aspect is the high sensitivity to possible adulteration of the result, which is associated with food intake shortly before blood sampling. In the last few decades, an alternative to blood tests in the assessment of the biomineral nutritional status has been the analysis of mineral content in head hair (Wójciak et al. 2014). This method has both advantages and disadvantages. The disadvantage of hair analysis is the sensitivity of the hair to environmental factors, hence hair used for analysis cannot be subjected previously to restorative hairdressing procedures like dyeing or permanent waving. Before the analysis, hair must also be thoroughly cleansed of hair care products (hair conditioners or shampoos).

An indisputable advantage is the non-invasiveness of this method. Hair grows about 1-1.5 cm per month, which means that when assessing 2-3 cm sections, the last few months of the nutrition of the subject are evaluated (Wójciak et al. 2014). Being dead tissues, hair can be stored for a long time and the time of chemical analysis is relatively short.

Taking all the above considerations together, the aim of the study has been defined as follows: to assess the content of selected minerals in the hair of elderly women, and to establish the relationship between the level of minerals in hair and their consumption in the daily food ration of these women.

SUBJECTS AND METHODS

Subjects

The study was carried out on 133 elderly women living in a nursing home. The inclusion criteria were based on the good physical and psychological condition of the subjects, and any women who took medication and/or supplements that could affect the study results were excluded from the study. 43 women did not meet these criteria. Finally, the study was based on 90 senior citizens, aged 78.4±7.3 years. The subjects were divided into three groups according to their age. The majority of the women lived alone (about 70% of the subjects), 16% registered light mobility problems, and 2% were immobilized. A full description of the subjects is presented in Table 1.

Anthropometric measurements

The weight and height of all subjects (wearing light clothing and no shoes) were measured using medical scales and a height meter to the nearest 0.1 kg and 0.1 cm, respectively. The BMI was calculated by dividing weight (kilograms) by height squared (square meter). The correct BMI was based on

Table 1

Characteristics of the population studied

Parameter		Total (n=90)	Age 65-74 (n=31)	Age 75-84 (n=35)	Age >85 (n=24)
Age (years) (range)		78.4±7.3 (65-93)	70.2±2.9 (65-74)	79.2±2.8 (75-84)	87.7±2.2 (85-93)
BMI (kg m^{-2}) (range)		25.3±4.2 (16.5-37.4)	26.3±3.8^{b} (18.1-37.4)	26.0±4.7^{b} (17.5-35.7)	23.2±3.5^{a} (16.5-28.6)
% of subjects	under/overweight	29/24	16/23	29/31	46/17
		chi-square test	χ^2=26.5; p<0.001		
Widows (% of subjects)		67.4	50.0	71.4	80.0
Mineral supplementation at least 3 months (% of subjects)		0	0	0	0
Reported cardiovascular problems (% of subjects)		25.0	22.0	28.0	25.0
Reported other age-related disorders including hypertension (% of subjects)		30.0	26.0	28.0	35.0
Mobility problems (% of subjects)		16.3	0	14.3	40.0
Bedridden/immobilized (% of subjects)		2.2	0	0	8.0

$^{a, a}$ – statistically significant differences at p<0.01

the criteria for elderly people (23.0-27.0 kg m^{-2}) presented by Hong et al. (2009) and Grzegorzewska et al (2016).

Mineral intake assessments

The mineral intake was calculated as the difference between the food rations served and the uneaten food residue. Dietary intake was based on the menu served to these senior citizens in their nursing home. Probable additional intake (including consumption of dietary supplements) and the residues were based on an individual nutritional interview with each subject over three preceding days (3x24-h recall), which was developed by the National Food and Nutrition Institute (Stanikowski et al. 2020). The amount of minerals in the daily food rations and residues were processed and evaluated using the Cambridge Diagnostics computer program “Aliant” based on the table of composition and nutritional value of food products (Kunachowicz et al. 2017).

Mineral concentration assessment

Previous publications by other authors have described the preparation of hair samples and the determination of minerals in hair, as well as reference values for hair elements (Wójciak et al. 2004, Chojnacka et al. 2010, Suliburska et al. 2011). The hair samples, taken from six different points of the occipital scalp (about 0.5 g, 1.0 cm from the skin), were washed three times in unionized detergent, deionized water, acetone, and deionized water again, then dried to dry mass (at 105°C). The wet mineralization in the nitric acid environment (65%, supra pure, Merck) using the microwave method (Milestone) was applied. After proper dilutions, the contents of calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu) and iron (Fe) in the hair samples were determined by flame atomic absorption spectrometry using a Zeiss AAS-3 spectrometer with a deuterium background correction. The accuracy of the method was verified by certified reference material (95-102%). The reference values were as follows (μg g^{-1} d.m): Ca – 600-1000, Mg – 40-60, Zn – 160-200, Cu – 10-20, Fe – 10-20.

Statistical analysis

The data are presented using descriptive parameters with arithmetic mean, standard deviation (SD), median, and range. Normality in frequencies was checked using the Shapiro-Wilk test. The data tested in this study are not from a normally distributed population. The Mann-Whitney test was used to establish the significance of differences between the groups for independent variables. The Spearman's correlation test was used to assess correlations between the parameters. A *p* value of less than 0.05 was taken as statistically significant.

The statistical power of the study with the number of subjects in each group was sufficient to achieve results at more than 80% probability. The data were analysed using the Statistica ver. 6.0 StatSoft computer programme.

RESULTS

The results obtained in this study are presented in Tables 1-5. Table 1 shows the Body Mass Index (BMI) of the subjects, in addition to the characteristics of the population. The mean BMI for senior citizens was in the correct range (25.3±4.2 kg m^{-2}), 29% of the subjects presented a BMI of less than 23 kg m^{-2}, and 25% were overweight (over 27 kg m^{-2}). The women aged 65-74 and 75-84 presented a similar mean BMI (about 26.0 kg m^{-2}), while the oldest women had a statistically lower BMI (23.2±3.5 kg m^{-2}; p<0.01). Statistically significant differences (p<0.001) were found in the distribution of BMI data in women of different ages. The fewest underweight women were in the youngest group of respondents (16%), while most were found among the oldest women (46%). Another relationship was noticed in the case of overweight women, with the most overweight women being aged 75-84 years (31%), while the lowest level was in those over 85 years old (17%).

Table 2 shows the intake of minerals with daily food rations (DFR) by all groups of respondents. The women, regardless of age, consumed similar

Table 2

Content of minerals in Daily Food Rations (DFR) in women according to their age (mg/person/day)

Parameter		Total (n=90)	Age 65-74 (n=31)	Age 75-84 (n=35)	Age >85 (n = 24)
Ca	arithmetic mean ±SD	737.8±346.3	688.3^{a}±320.5	804.7^{a}±350.3	704.2^{a} ±370.7
	median	708.0	675.0	786.0	577.4
	range	227.7-1672.9	244.9-1488.3	242.0-1557.9	227.7-1672.9
	% of RDA* and EAR**	61.5	57.3	67.1	58.7
Mg	arithmetic mean ±SD	259.8±97.2	248.7^{a}±100.2	255.8^{a}±103.9	279.8^{a}±83.0
	median	244.0	231.1	232.4	282.9
	range	67.9-450.6	93.3-450.6	67.9-427.6	167.5-450.6
	% of RDA and EAR	81.2	77.7	79.9	87.4
Zn	arithmetic mean ±SD	7.15±2.93	7.32^{a}±3.36	7.49^{a}±3.09	6.43^{a}±1.90
	median	6.90	6.90	7.30	6.64
	range	3.00-14.90	3.00-14.10	3.11-14.90	3.45-9.35
	% of RDA and EAR	89.3	91.4	93.6	80.3
Cu	arithmetic mean ±SD	0.85±0.37	0.89^{a}±0.41	0.84^{a}±0.36	081^{a}±0.32
	median	0.73	0.78	0.73	0.73
	range	0.24-2.23	0.24-2.23	0.24-1.75	0.50-1.75
	% of RDA and EAR	94.5	99.3	93.6	89.7
Fe	arithmetic mean ±SD	6.71±2.29	6.73^{a}±2.51	7.06^{a}±2.34	6.16^{a}±1.86
	median	5.91	5.87	6.20	5.57
	range	4.05-12.59	4.09-12.59	4.40-12.10	4.05-10.45
	% of RDA and EAR	67.1	67.1	70.6	61.6

* RDA – recommended daily allowances
** EAR – Estimated Average Requirement
$^{a, a}$ – non-significant differences between means at p<0.05
Normality in frequencies was checked using the Shapiro-Wilk test. The data tested in this study are not from a normally distributed population. The Mann-Whitney test was used to establish the significance of differences between the groups for independent variables. The Spearman's correlation test was used to assess correlations between the parameters.

amounts of Ca, Mg, Zn, Cu, and Fe (mean 737.8, 259.8, 7.15, 0.85, and 6.71 mg/person/day, respectively). The smallest coverage of the recommended daily allowance was demonstrated for Ca (mean 61.5% for all subjects, and 58.7% for the oldest ladies) and Fe (mean 67.1% for total subjects, and 61.6% for the oldest ladies). However, the daily Cu intake was mostly on the correct level in the diet of all elderly women (94.5% of Recommended Daily Allowances RDA and Estimated Average Requirement EAR (Jarosz et al. 2020), while the Zn intake was on the mean level at almost 90% of the RDA and EAR for all subjects, but for the oldest ladies the RDA and EAR was covered in 89.7% for Cu and 80.3% for Zn. The Mg intake in all subjects covered 81.2% of the RDA and EAR, while the oldest women ate the daily food rations with Mg on the level of 87.4% of RDA and EAR (77.7% of RDA and EAR for the youngest women).

The level of hair minerals in senior citizens according to their age is presented in Table 3. There were no statistically significant differences between the groups in terms of the mean level of Ca, Mg, and Cu in the hair of women according to their age (about: 637.9, 37.37, and 11.47 μg g^{-1} d.m., respectively). The percentage distribution of subjects according to the reference values did

Table 3

Levels of minerals in hair of seniors according to age (μg g^{-1} d.m.)

Parameter		Total (n=90)	Age 65-74 (n=31)	Age 75-84 (n=35)	Age >85 (n=24)
Ca	arithmetic mean ±SD	637.9±367.9	664.6^{a}±362.3	683.6^{a}±369.4	602.3^{a}±385.6
	median	522.6	565.1	518.1	472.0
	range	137.3-1629.3	137.3-1336.7	138.6-1448.2	143.3-1629.3
	below/above RV's* (%)	29/20	26/22	29/20	33/17
	chi-square test		not significant		
Mg	arithmetic mean ±SD	37.37±21.25	40.21^{a}±22.16	36.00^{a}±20.34	35.71^{a}±21.87
	median	29.6	31.99	28.66	27.22
	range	16.67-118.3	19.33-105.4	18.50-118.3	16.67-97.89
	below/above RV's (%)	75/16	71/19	80/11	75/17
	chi-square test		not significant		
Zn	arithmetic mean ±SD	186.2±62.8	204.5^{b}±63.3	183.8^{a,b}±60.5	166.2^{a}±61.1
	median	188.8	193.5	191.2	153.4
	range	65.7-346.4	65.7-346.4	77.3-331.4	65.7-264.3
	below/above RV's (%)	39/40	29/48	37/37	54/33
	chi-square test		χ^2=15.7; p<0.01		
Cu	arithmetic mean ±SD	11.47±5.54	12.16^{a}±6.03	11.21^{a}±5.75	10.97^{a}±4.61
	median	10.34	10.60	10.23	10.29
	range	3.60-33.06	3.61-33.06	4.23-27.06	3.60-21.64
	below/above RV's (%)	44/8	45/7	46/11	42/4
	chi-square test		not significant		
Fe	arithmetic mean ±SD	17.28 ±12.48	22.26^{b} ±13.54	18.16^{b} ±12.62	9.59^{a} ±5.60
	median	13.73	21.14	14.27	7.69
	range	1.95-69.08	6.30-55.63	4.56-69.08	1.95-21.49
	below/above RV's (%)	32/34	16/55	26/34	58/8
	chi-square test		χ^2=64.9; p<0.001		

* RVs-reference values

^{a,a} – non-significant differences between means at p<0.05

^{a,b} – statistically significant differences at p<0.05

not differ between the age groups for these minerals. 1/3 of the women had a level of hair Ca below, while 1/5 had it above the RVs. On average, 75% of women presented the level of Mg in the hair below the RVs, and 16% above RVs. 44% of the subjects presented a hair Cu concentration below the RVs and 8% of the women had a Cu level above these values.

However, the mean level of Zn in the hair of the women was in the range of RVs (186.2 μg g^{-1}d.m.), and almost 40% of the women presented a level of hair-Zn below and above the RVs (Table 3). Statistically significant differences between age groups of women regarding the level of hair-Zn, as well as the distribution of subjects according to the RV range were found in the study. The oldest ladies showed a statistically lower level of hair-Zn (166.2±61.1 μg g^{-1} d.m.) than the youngest ones (204.5±63.3 μg g^{-1} d.m.). The percentage distribution of the population in relation to the range of RVs confirms this observation, and differed significantly between the age groups (chi^2=15.7, p<0.01). The proportion of women with the lowest content of Zn in the hair increased with age, and was below RVs in 29% of the youngest women, through 37% in the group aged 75-84 years, to 54% in the oldest group.

A similar situation was observed in the case of the Fe hair level. The ladies above 85 years of age presented a significantly lower Fe mean concentration in their hair (9.59±5.60 μg g^{-1} d.m.) than the groups of women aged 65-74 and 75-84 years (22.26±13.54 and 18.16±12.62 μg g^{-1} d.m., respectively). The distribution in relation to the RV range also showed statistically significant differences between the age groups (chi^2=64.9, p<0.001). Only 16% of the women in the youngest group showed the Fe concentration in the hair below RVs, and 55% of the results were above these values, whereas in the oldest group as many as 58% of women presented a hair-Zn level below RVs and only 8% above. In the 75-84 age group, these proportions were the following: 26% vs. 34%, respectively.

A comparison of ratios between hair mineral concentrations is presented in Table 4. The mean Ca/Mg, Zn/Cu, Zn/Fe, and Fe/Cu ratios in the hair of all subjects were as follows: 19.37, 19.70, 16.87, and 1.63, respectively. There were no statistically significant differences between aged-divided groups of women in relation to Ca/Mg and Zn/Cu ratios. The Zn/Fe ratio in the subjects' hair was significantly higher in women over 85 years old than in younger women (23.63 vs. ca. 14.50), whereas the Fe/Cu ratio was significantly lower in this group than in the others (0.97 vs. ca. 1.80).

Table 5 shows significant correlations between the data. Significant correlations were found between age and Zn and Fe concentrations in hair (r=-0.231, p<0.05 and r=-0.422, p<0.001, respectively). The BMI of the women correlated positively with the hair-Ca level (r=0.206, p<0.05) and hair-Cu level (r= 0.277, p<0.01). The Ca intake by women correlated with the hair-Zn level (r=-0.219, p<0.05), as well as the Zn intake with the concentration of Ca in the hair of study population(r=-0.283, p<0.01).

Table 4

Comparison of ratios between elements in the hair of subjects

Parameter		Total (n=90)	Age 65-74 (n=31)	Age 75-84 (n=35)	Age >85 (n=24)
Ca/Mg	arithmetic mean ±SD	19.37±12.13	17.35^{a}±8.98	20.40^{a}±14.08	19.64^{a}±12.73
	median	16.40	17.22	16.54	15.76
	range	4.01-63.00	4.28-46.04	4.26-63.00	4.01-49.72
Zn/Cu	arithmetic mean ±SD	19.70±11.08	20.21^{a}±10.70	20.84^{a}±12.79	17.06^{a}±7.88
	median	18.38	18.25	19.53	18.24
	range	4.01-56.75	4.31-48.35	4.01-56.75	6.32-38.92
Zn/Fe	arithmetic mean ±SD	16.87±12.97	14.10^{a}±10.55	14.90^{a}±10.37	23.63^{b}±16.37
	median	12.55	11.91	12.00	17.45
	range	1.67-69.33	2.55-40.62	1.67-43.45	4.06-69.33
Fe/Cu	arithmetic mean ±SD	1.63±1.04	1.95^{b}±1.18	1.71^{b}±0.92	0.97^{a}±0.56
	median	1.37	1.54	1.61	1.02
	range	0.18-5.57	0.39-5.57	0.44-3.67	0.18-2.21

$^{a, a}$ – non-significant differences between means at p<0.05
$^{a, b}$ – statistically significant differences at p<0.05
Normality in frequencies was checked using the Shapiro-Wilk test. The data tested in this study are not from a normally distributed population. The Mann-Whitney test was used to establish the significance of differences between the groups for independent variables. The Spearman's correlation test was used to assess correlations between the parameters.

Considering the division according to age, in women over 85 years of age there was an explicitly proportional statistically significant relationship between the BMI and Ca content in hair (r=0.472, p<0.05), while in women aged 75-84, such a significant relationship was between the BMI and the level of copper in the hair (r=0.441, p<0.01). The higher copper intake in DFR significantly influenced lower levels of magnesium in the hair of the oldest women (r=-0.485, p<0.05), while in the group of youngest women (under 74 years) there was a significant (r=-0.384, p<0.05), inversely proportional relationship between calcium intake in DFR and zinc content in the women's hair. A similar relationship was observed in this group between the consumption of zinc in DFR and the calcium content in senior citizens' hair (r=-0.378, p<0.05).

DISCUSSION

Western society is ageing. The increasing number of older people in the population is starting to be seen as a challenge that is largely connected with providing a decent, healthy life for the elderly. Although the ageing process cannot be stopped, it can be slowed down. This phenomenon has been

Table 5

Significant correlations between the data

Parameter		*R*	*P*
In all subjects			
Age/ Age/	hair-Zn hair-Fe	-0.231 -0.422	0.028 0.000
BMI/ BMI/	hair-Ca hair-Cu	0.206 0.277	0.049 0.008
DFR-Ca/	hair-Zn	-0.219	0.038
DFR-Zn/	hair-Ca	-0.283	0.007
According to the age			
In women >85 y.o			
BMI/	hair-Ca	0.472	0.021
DFR-Cu/	hair-Mg	-0.485	0.016
In women 75-84 y.o.			
BMI/	hair-Cu	0.441	0.008
In women <74 y.o.			
DFR-Ca/	hair-Zn	-0.384	0.033
DFR-Zn/	hair-Ca	-0.378	0.036

The Mann-Whitney test was used to establish the significance of differences between the groups for independent variables. The Spearman's correlation test was used to assess correlations between the parameters.

observed in recent years, when life expectancy has been increasing. An early response to the symptoms of age-related diseases and appropriate treatment are very important in this area (Maki et al. 2016). Adequate nutrition for the elderly is of special importance in this respect. A strong relationship between nutritional status and the progress of the ageing process has been repeatedly demonstrated. Both obesity and severe malnutrition cause a decline in the quality of life of older people, impeding the process of treatment and convalescence after illness, as well as leading to premature death (Jousilathi et al 1999). Proper supply of essential nutrients becomes difficult due to disturbances in absorption and digestion, as well as concomitant diseases and drug use, which may interact with food ingredients and additionally limit their availability. All these processes should be taken into account when planning nutrition for the elderly.

Food components that should be particularly addressed in the nutrition of the elderly are micronutrients, such as minerals. Trace elements, as exclusively exogenous ingredients, should be provided in their entirety with a diet. Both their insufficient intake and excess in food can lead to a number of disorders (Kim et al. 2013). Minerals play a significant role in a number of important processes in the human body (Kristensen et al. 2015, Miki et al.

2015, Głąbska et al. 2016). Their role as cofactors of enzymes and their importance in oxidation processes elevates them in terms of ingredients that prevent ageing processes. It is believed that their special role in the ageing process makes deficiencies of minerals responsible for many age-related diseases, such as iron deficiency, anaemia, osteoporosis, type II diabetes, muscular dystrophy, etc. (Giorgini et al. 2017). Along with irregularities in the mineral metabolism of the body, there is also an abnormal functioning of the nervous system, the occurrence of dementia, depression, cognitive disorders, including memory loss, etc.

On the other hand, because knowledge on this subject is common, it leads to excessive, often uncontrolled supplementation of minerals among the elderly. Although there is little information about supplementation in the oldest group in society, some authors are of the opinion that 50-70% of adults take dietary supplements (Kałużna et al. 2004, Blumberg et al. 2017). Wójciak et al. (2019) asked almost 100 elderly women about using their use of dietary supplements, and all of them gave a positive answer. Other Polish authors reported that the supplements in Poland are over-consumed (Kocylowski et al. 2018). This observation is similar to the one presented by a PolSenior population study reported by Bogusz et al. (2013), who conducted a study of physical health in older people.

This controversy suggests that research into the nutrition and nutritional status of seniors is still necessary, the more so that the uptake of minerals with the diet of older women is insufficient, and supplementation often incorrectly selected. There are no unambiguous data in the Polish and world literature assessing the uptake of minerals in the diets of healthy elderly women. The authors usually focus on other age groups or disease states. From such data, it is possible to collect information about old people in the control groups presented in it. However, there are no comparisons regarding women over 80 years of age. The results reported in this paper show that the intake of Ca, Mg, Zn, Cu and Fe in the group of elderly women was insufficient and at a similar level in every age group. The lowest intake was observed for Ca and Fe (at ca 60% of RDA and EAR) and the highest for Mg, Zn and Cu (ca 80% of RDA and EAR). However, similar results for Ca and Fe intake in the diets of Polish women in reproductive age were observed by Suliburska (2011), and the Mg, Zn, and Cu intake in this group was almost RDA or above it. Głębska et al. (2016) showed similar results to those obtained in this study, when analysing a sub-population of postmenopausal Polish active working women above 50 years old, based on a large cohort study of over 400 participants. The Ca intake in the diets of these women was about 50-55% of RDA, and the Mg intake stood at 80-90% of RDA. This recommendations concerned consumption of Zn, Cu, and Fe. In comparison to the other European countries, the data presented by Kristensen et al. (2015), which were obtained from a Danish National Survey of Dietary Habits and Physical Activity study on over 600 women aged 33-52, suggested that the intake of Ca, Mg, and Zn in this group was about 20% above the recommendations, while the Fe intake was only 50% of the norms. This deficit was supplemented to the normative level of intake. Kocylowski et al. (2018) also reported an insufficient intake of Ca (84% of RDA, EAR), Mg (75% of RDA, EAR), Zn (91% of RDA, EAR) and Fe (39% of RDA, EAR) in the diets of pregnant women. However, these deficits were compensated with supplementation to the level of 89, 91, 116, and 112% of RDA and EAR, respectively. In these studies, elderly women were not supplemented with minerals.

Assessment of the mineral nutritional status in elderly people is hampered by the problem of sampling. Traditionally, blood analysis or evaluation of the content of elements in urine is used. However, minerals are rarely evaluated without obvious symptoms of their deficiencies. Due to the processes of homeostasis, such symptoms appear only in connection with significant irregularities in the metabolism of minerals, leading to osteoporosis, anaemia or other related disorders. It is extremely important that deficiencies of minerals are detected as soon as possible, which could enable the implementation of changes in nutritional habits or supplementation. In recent years, hair analysis has been used for this purpose. Hair sampling is non-invasive, painless and can be done relatively often. In the last few decades, there have been many studies on the content of minerals in the hair of different groups (Blaurock et al. 2014). However, not many studies can be found that concern the content of minerals in the hair of old women and changes in their level caused by age.

In this paper the Ca, Mg, Zn, Cu, and Fe concentrations in the hair of women above age 65 were examined. Many publications (Kim et al. 2011, Wiechuta et al. 2012) show significant differences in calcium content in the hair of women in different age groups. Women of reproductive age usually have this element at the level of several thousand micrograms per gram, whereas in women in the postmenopausal period, the calcium content is reduced to several hundred. The content of calcium at later age does not change. Similarly, in this study, the level of this element in the hair did not differ in the groups of women of different age, same as the proportion of women with a low and elevated (relative to reference values) level of calcium in their hair (about 1/3 of the subjects).

No such dependence was noted in the experiments concerning the magnesium content in hair. Most often, the level of this element is similar in different age groups of women and was about 100 micrograms per gram of hair. This level is twice as high as that in hair of the elderly women presented in this work. On average, irrespective of age, the women had a low magnesium concentration of about 50 micrograms, which corresponded to the levels characteristic of some disorders. Park et al. (2017) reported that the level of magnesium in the hair of 72 women with a mean age of 62 years with coronary artery calcification was ca 50 $\mu g\ g^{-1}$ and was significantly lower than in healthy subjects. A similar level of hair Mg was reported by Kim et al. (2011) in 44 women (mean age 44) with fibromyalgia (52 $\mu g\ g^{-1}$) in comparison to the control group (72 μg g^{-1}). The lower level of hair Mg in hypertensive postmenopausal women in comparison to healthy women was also reported by Gonzales-Munoz et al. (2010). The data presented in this study and in the cited articles suggest that a low level of magnesium in hair in older women may result from the course of unrecognized disease processes. This may be related to the occurrence of approximately 25% of the elderly patients with disorders of the cardiovascular system and other age-related disorders, including hypertension (in about 30% of subjects). Park et al. (2017) are also of the opinion that among middle-aged and elderly women without clinical cardiovascular disease, the association between coronary artery calcification and hair calcium/magnesium ratio is stronger in those with a higher ratio than in those with a lower ratio. The elderly women participating in this study were characterized by a relatively higher calcium/ /magnesium ratio in hair (about 20) compared with the results calculated on the basis of the reference values (15-17) and those presented in other works on younger and healthy women (10-17) (Kim et al. 2013, Park et al. 2017, Kunachowicz et al. 2017). Similarly, Gonzales-Munoz et al. (2010) found a statistically significant higher level of hair Ca/Mg ratio in hypertensive postmenopausal women in comparison with the normotensive group (17 vs. 10). On the other hand, some authors found much a higher hair Ca/Mg ratio in young, 20-year-old women than in those presented in this study, at the level 22-27 (Park et al. 2010, Chojnacka et al. 2010, Kocylowski et al. 2018). This was mainly due to the fact that the calcium content in hair was above 2000 μg g^{-1} in these studies, whereas in other research it did not exceed 1000 μg g^{-1}. Such differences in the content of calcium in hair, which are associated with age, are also suggested by other authors (Wójciak et al. 2004, Suliburska 2011, Skalny et al. 2018). They are attributed to the intensification of osteomalacia and osteoporosis in postmenopausal women (Jousilahti et al. 1999, Ignasiak et al. 2016, Blumberg et al. 2017).

Chojnacka et al. (2010) sought the reference values for trace element concentrations in the hair of humans, and state that the average copper content in students' hair is at the level of 21.5 μg g^{-1} d.m., and is twice as low in men as in women (ca. 13 vs. 26 μg g^{-1} d.m.). It is also lower in women's undyed hair than in dyed hair (23 vs. 32 μg g^{-1} d.m.). Although the contamination of hair with hair dyes is known, the use of dyed hair for analysis of mineral status is not recommended. However, Suliburska (2011) found similar results to ones reported by Chojnacka et al. (2010) in terms of hair copper concentrations in women aged 25 years, in that the older women (above 30 years) in her study had a significantly lower level of this metal in their hair (ca 13 μg g^{-1} d.m.) Kim et al. (2013), after analysing trace elements and toxic metals in the hair of Korean women of different ages (20-60 years of age), were of the opinion that the hair copper concentration is not age-related. This data correspond with the present study, where the level of copper concentration in elderly women was similar in different age groups.

Some authors (Wójciak et al. 2004, Chojnacka et al. 2010, Gonzales-Munoz et al. 2010) believe that the level of copper in hair is not as important as the relationship between copper and zinc in hair in an assessment of this metal status in organisms. Gonzales-Munoz et al. (2010) are of the opinion that the Zn/Cu ratio is statistically significantly higher in hypertensive postmenopausal women than in normotensive ones, which is associated with the finding that the levels of zinc in hair and other tissues decrease with arterial hypertension. Other authors presented similar higher levels of Zn/Cu ratio in the hair of women with different kinds of disorders in comparison to healthy controls (Rodenas et al. 2011, Bossola et al. 2014, Błażewicz et al. 2017, Skalny et al. 2018). One interesting observation, although not discussed, was made by Kim et al. (2011) in different age groups of pregnant women. Although the level of Cu in the hair of those women did not differ, older women presented a mean hair Zn concentration significantly higher than younger subjects, hence the Zn/Cu ratio was also higher in this group. Those authors found a lower Zn/Cu ratio in the hair of underweight, overweight and obese pregnant women than in women with normal weight. This data corresponds to that obtained in this work. Although we did not find any significant differences in hair Cu concentration between elderly women in different ageing groups, the oldest women had a significantly lower Zn concentration than the younger groups, hence the Zn/Cu ratio in the oldest ladies was significantly lower than in women younger than 84 years.

The lowest mean level of iron in the hair of women over 85 years was observed in the present study, and it was half that of younger groups. Almost 60% of the oldest women presented a hair iron concentration below the reference values, while ca 20% of the younger women were below these values. The mean hair Fe concentrations same as the level obtained in this study from hair from the group of women less than 84 years old were found by others (Hong et al. 2009, Kim et al. 2011, Błażewicz et al. 2017). Suliburska (2011) observed a statistically significant decrease in the hair Fe concentration in women 31-40 years in comparison to those below this age, although also above this age. On the other hand, the low levels of hair iron is usually presented in women using restricted diets such as food deprivation, vegetarian, and low calorie diets (Wójciak et al. 2004, Wójciak 2014). It can be assumed that this condition concerns changes in the body leading to iron deficiency anemia, which is especially dangerous in women. Low iron levels also lead to depressed mood and depression, as some authors point out, as well as zinc deficiency in hair, as found in this study. Changes in shortages of one element are associated with the excessive occurrence of others. This shows statistically significant differences between the ratio of elements in the hair found in this work, which may indicate adverse changes in the body of older people, associated with oxidative processes, especially in microelements.

One of the most important aspects of research on the trace element status in humans is the search for a relationship between the daily intake of biometals and the nutritional and health status of populations. Many researchers are striving to find such relationships. Hong et al. (2009) studied Korean female adults (mean age = 41 years) and found a positive correlation between age and hair sodium, chromium and sulphur, and a negative one with zinc. This observation is similar to those presented in this study. We also found age-related inverse changes in hair zinc and iron levels. In many studies, similar relationships can be found that could be the cause of disorders accompanying iron deficiency anaemia and ones due to by zinc deficiencies. A significant correlation between age and the hair zinc concentration was also found by Skalny et al. (2018) in their study on pregnant women. In her work on the relationship between daily metals intake and hair concentration of elements, Suliburska (2011) found an inverse relationship only between copper in hair and the content of calcium, magnesium, iron, and zinc in daily diets of the women studied. Although no such relationships were found in the present study, a strong negative correlation was observed between daily calcium intake and zinc hair level, as well as daily zinc intake and calcium concentration in the hair of elderly women, especially in the oldest ladies This may have resulted from the competitive role of both elements. Excessive intake of calcium in a diet can cause the absorption of microelements, including zinc, in human bodies. Similarly, increasing amounts of zinc in a diet negatively affect calcium management. Some authors observed this effect even in women who had been given calcium supplementation (Rodens et al. 2011, Wójciak 2014), when the serum calcium concentration was stable. An interesting effect was observed concerning the positive relationship between total nutritional status, expressed by the BMI, with calcium and copper concentration in hair, but not in women below 74 years old. It seems that the general nutritional status is more important in shaping the trace element status in the circumstances of food deprivation and malnutrition than in over-nourishment. In our study, a significant percentage of women over 75 years of age were characterized by a lower than normal BMI, as well as the zinc and iron concentrations in their hair being under the reference values.

CONCLUSIONS

Generally, the results of the current study demonstrate that increased age is associated with a significant change in hair trace element content in elderly women, especially those over 85 years old, which is associated with an increasing number of undernourished women in this age group. It seems that this is not due to a different elemental intake from a diet, nor from the course of the ageing process itself. However, much more research is still needed to replicate the results and to identify the underlying causes. Gaining better insight into age-related changes in trace metal levels may help to prevent adverse age-related symptoms, including those related to element deficiencies, and increase the quality of life for elderly people. On the other hand, considering a high range of deficiencies for example shown in this study, research on the trace element status in the elderly may result in a better fit of the necessary supplementation at this age.

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DECLARATIONS

Authors' Contribution

Conceptualization, R.W.W.; methodology, A.C.W. and R.W.W.; formal analysis, R.W.W.; investigation, A.C.W. and R.W.W.; writing-original draft preparation, R.W.W., A.C.W. and K.M.; writing-review and editing, R.W.W., K.M. and Z.K. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

All the study procedures were conducted in accordance with the Declaration of Helsinki, and the Bioethics Committee at the Medical University of Poznań approved the protocol (approval no. 184/18, 2018).

Informed Consent Statement

The subjects were informed of the research's purpose and procedure and provided written, informed consent to participate in the study.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to their containing information that could compromise the privacy of the research participants.

Conflicts of Interest

The authors have no conflict of interest to declare.