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Chromium, Zinc and Magnesium Concentrations in the Pubic Hair of Obese and Overweight Women

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Abstract The study addressed chromium, zinc and magnesium concentrations in the pubic hair of obese and overweight women. It was carried out on hair collected from 85 women at the age of 16–80 living in the Podkarpackie Voivodeship (southern Poland). The experimental and control groups consisted of 39 and 46 females, respectively. The pubic hair was prepared under a procedure established by the International Atomic Energy Agency, followed by wet digestion in a microwave oven. The concentration of the metals in the pubic hair and reference material was assayed with the flame (Mg, Zn) and flameless (Cr) atomic absorption spectrometry. The pubic hair of overweight and obese women from the experimental group revealed significantly higher chromium and magnesium concentrations and significantly lower concentrations of zinc than in the control group. An increase in BMI brought about an increase in chromium and magnesium concentrations while zinc concentration decreased with increasing BMI. The disturbances in the mineral balance of overweight and obese women were also demonstrated by significantly different ratios of the elements compared with the control group.

Keywords Pubic hair · Chromium · Magnesium · Zinc · Overweight · Obesity

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Introduction

Obesity is a disease in which the amount of excess fat tissue is big enough to affect adversely human health. It is becoming a worldwide problem and is regarded as one of the most serious threats to our health [1, 2]. The incidence of obesity reflects changes in modern society whose lifestyle is very often based on the consumption of high-calorie food and decline in the opportunity and motivation for physical activity [3]. At present, over one billion people are estimated to be overweight out of which 300 million suffer from obesity. Being overweight is more common among males while obesity among females.

Obesity is characteristic of increased content of fat [4], therefore its definition should be based on the amount of fat tissue in a human body. However, it is hard to estimate it accurately and thus obesity is defined using body mass index (BMI) which includes both body mass and height: BMI = weight (in kilograms)/height (in metres)^2.

BMI correlates strongly with the amount of fat in the organism and is very useful for epidemiological purposes [5]. The classification of obesity based on BMI adopted by WHO is as follows: 18.50–24.99 kg/m^2—normal range, ≥25.00 kg/m^2—overweight, 25.00–29.99 kg/m^2—pre-obese and ≥30.00 kg/m^2—obesity (30.00–34.99 kg/m^2—obesity class I, 35.00–39.99 kg/m^2—obesity class II, ≥40.00 kg/m^2—obesity class III) [5]. The interpretation of BMI should take into account age and gender, because women have a higher percentage of fat in their organs than men due to their lower weight of muscles and bones. Moreover, older people tend to have a higher percentage of fat tissue than young people because the composition of their bodies changes with age [6].

Overweight and obese people suffer from metabolic disorders and often lack a lot of important elements. Chromium is one of the main trace elements that participate in metabolic processes. It plays a very important role in the metabolism of carbohydrates, lipids and proteins, increasing insulin activity in particular [7–9]. An insufficient amount of chromium can cause plenty of physiological disturbances which increase the risk of contracting cardiovascular diseases or diabetes. Zinc and magnesium are elements indispensable to the human organs. Zinc is essential for the metabolism of hormones, such as insulin and thyroid hormones. It is also active in the metabolism of glucose and lipids and acts as a cofactor for numerous enzymes. Thus, its deficiency is closely related to insulin resistance, hyperglycemia and impaired glucose tolerance [10]. As to magnesium, it performs a lot of physiological and homeostatic functions, maintains stable metabolism, prevents cardiovascular diseases and regulates blood pressure [11].

Trace elements can be assayed in hair which is a very good diagnostic material with the capability to bind metals, depending on the presence of cysteine, a protein with chelating properties. The concentrations of elements are much higher in hair than blood, which makes assays easier. Besides, hair provides data on metal concentrations over a longer period of time, i.e. approx. 1–2 months. The elemental analysis of hair is used to assess protracted exposure to toxic metals [12, 13]. Its disadvantage is that it is difficult to eliminate the impact of external contaminants and identify accurately the main sources of elements in hair connected to, e.g. environmental contamination. Carrying out research on a homogeneous group, using pubic hair and employing a suitable washing procedure considerably reduce those impacts. Since hair is a good indicator of nutrition state, its tissues also reveal elemental disturbances in obesity. This study determined the concentrations of chromium, zinc and magnesium in the hair of overweight and obese women with normal BMI and addressed the question of how obesity and overweight affect the mineral composition of hair.

Materials and Methods

The study has been approved by the Bioethics Committee of the Medical University of Silesia in Katowice. The pubic hair samples were taken from women living in the Podkarpackie Voivodeship (southern Poland). They were all asked to complete a questionnaire on their age, height, weight, living standards, medications taken, diseases, diet, occupational hazard to metals or smoking habits. The results excluded women who had been taking medications or diet supplements which contained the metals in question for the last 3 years prior to the tests. Those who neither smoked nor suffered from chronic diseases, or were not occupationally exposed to the metals were accepted. Altogether, 85 women took part in the research and were divided into two groups—experimental and control ones. The criterion for the division was BMI. The experimental group contained 39 women at the age of 23–80 (42.6±12.8 on average). They were subdivided into two subgroups—those overweight (30.0>BMI≥25.0) and obese (BMI ≥30.0). The former contained 25 females aged 23–66 (41.2±10.4 on average) while the latter comprised 14 women aged 30–80 (43.5±15.9 on average). Forty-six women, aged 16–55 (38.0±9.5 on average), belonged to the control group. The women from both experimental and control groups were also divided into four age groups. The first one included women below 29 years of age, the second between 30 and 39, the third between 40 and 49, and finally the fourth group over 50.

The collected pubic hair was prepared following a procedure established by the International Atomic Energy Agency [14]. The hair was washed with deionized water and pure acetone. The hair samples were rinsed with acetone, then three times with water followed by acetone again. Each time, the contact time with the solutions was about 10 min. After being dried at 105°C to constant mass, uniform weight (about 200 mg) of hair samples was digested in a microwave, using 2 mL of concentrated spectrally pure HNO_3. After digestion, the acid was vaporized out of the samples, 0.5 mL of HNO_3 and 10 mL of water were added and then the content was transferred into 25 mL measuring flasks and filled to volume.

Apart from the pubic hair, digestion was simultaneously carried out on CRM 397 reference material (Trace Elements in Human Hair, Community Bureau of Reference). The results from six runs were as follows: $Zn_{certified}$ 205 μg/g, $Zn_{assayed}$ 199 μg/g, $Cr_{certified}$ 0.37 μg/g and $Cr_{assayed}$ 0.38 μg/g. The accuracy of magnesium assays was verified using the method of standard addition.

The concentrations of metals in the pubic hair and reference material samples were assayed with the flame (Mg, Zn) and flameless (Cr) atomic absorption spectrometry, using Varian SpectrAA 880 and SpectrAA 880Z spectrophotometers. The concentrations of the elements in the pubic hair were determined following commonly acknowledged measurement procedures.

The results were statistically analysed with Microsoft Excel and Statistica ver. 9. The distribution of the data sets considerably differed from the normal one, therefore the median was used in the discussion. The testing of the statistical variability of the differences between the groups was based on the Mann–Whitney *U* test (two groups) and Kruskal–Wallis one-way analysis of variance (several groups).

Results

The concentrations of chromium, zinc and magnesium in the female pubic hair are given in Table 1. The median of

Table 1 Metal concentrations in the pubic hair of women in the experimental and control groups (in micrograms per gram)

Parameter	Experimental group			Control group (n=46)	Results of the U Mann–Whitney test (p value)
	Total (n=39)	Overweight (n=25)	Obesity (n=14)		
Cr					
Average	0.17	0.16	0.19	0.15	0.02
Standard deviation	0.06	0.05	0.06	0.07	
Median	0.17	0.16	0.18	0.14	
±95% CI	0.05–0.07	0.04–0.07	0.04–0.10	0.06–0.09	
Lower quartile	0.13	0.12	0.15	0.10	
Upper quartile	0.22	0.20	0.24	0.17	
Minimum	0.08	0.08	0.10	0.06	
Maximum	0.31	0.29	0.31	0.45	
Mg					
Average	216.92	172.92	295.49	108.15	0.00
Standard deviation	193.22	168.40	215.43	80.16	
Median	171.49	111.53	279.96	86.22	
±95% CI	157.91–249.02	131.49–234.27	156.17–347.06	66.49–100.97	
Lower quartile	59.66	58.05	153.43	54.98	
Upper quartile	313.60	192.87	336.77	123.01	
Minimum	38.10	38.10	40.71	22.66	
Maximum	837.07	702.23	837.07	370.20	
Zn					
Average	174.53	164.98	191.58	194.49	0.01
Standard deviation	59.33	42.83	80.10	54.74	
Median	163.30	160.70	165.05	194.10	
±95% CI	48.49–76.47	33.45–59.59	58.06–129.04	45.40–68.94	
Lower quartile	137.40	146.10	132.80	152.90	
Upper quartile	174.10	172.10	188.60	216.00	
Minimum	97.60	97.60	120.60	85.10	
Maximum	343.35	337.20	343.35	340.10	

chromium concentrations in the experimental group was 0.17 μg/g, while the value for the control group was lower—0.14 μg/g (Table 1). The difference in chromium concentrations in the hair of the women from the control and experimental groups was statistically significant (Mann–Whitney U test, p=0.02). On the other hand, the median of chromium concentration in the pubic hair of the overweight women reached 0.16 μg/g and was insignificantly lower compared with the obese women (Table 1). The median concentration of magnesium in the hair of all the tested women was 158.05 μg/g. In the experimental group, the median reached 171.49 μg/g, being 86.22 μg/g in the control group. The difference was statistically significant (Mann–Whitney U test, p=0.00). A significant difference was also found while comparing the concentrations of magnesium in the hair of overweight and obese women; the medians were 111.53 and 279.96 μg/g, respectively. The medians of zinc concentrations in the hair of the women in the experimental and control groups were 163.30 and 194.10 μg/g, respectively. The difference in zinc concentrations in the hair of the women from the control and experimental groups was statistically significant (the Mann–Whitney U test, p=0.01). The median of zinc concentration in the pubic hair of the overweight women was much lower than obese women and reached 160.70 and 165.05 μg/g (p=0.78), respectively (Table 1).

The concentrations of the metals in the pubic hair of the women from the experimental and control groups, including the age division, have been shown in Table 2. In the experimental group, the highest medians were found for 40–49-year-old women, i.e. the third age group, the lowest being observed for the youngest women, i.e. below 29 years of age. In the control group, the highest medians for chromium were found for 30–39-year-old women. No significant difference was spotted for chromium concentrations in the

Table 2 The medians and 95% CI of metal concentrations in the pubic hair of particular age groups (in micrograms per gram)

Age	*n*	Cr	Mg	Zn
Experimental group				
<29	3	0.15	175.25	156.70
		0.03–0.31	37.16–322.35	44.44–239.83
30–39	10	0.17	191.07	161.15
		0.12–0.21	85.83–487.12	130.22–216.81
40–49	13	0.20	89.97	159.30
		0.15–0.23	72.89–199.27	134.32–203.23
>50	13	0.17	201.87	165.90
		0.14–0.19	152.06–370.40	148.53–228.53
Control group				
<29	24	0.14	98.56	207.20
		0.12–0.16	80.60–153.06	176.27–228.22
30–39	10	0.15	84.53	194.10
		0.11–0.16	51.03–123.05	164.76–245.86
40–49	9	0.14	82.68	165.20
		0.07–0.25	35.41–187.06	150.56–192.79
>50	3	0.14	57.20	148.40
		0.11–0.17	32.18–187.19	69.83–259.90

pubic hair of the women from particular age groups, both in the experimental (Kruskal–Wallis one-way analysis of variance, $p=0.64$) and control ($p=0.99$) groups. In the experimental group, the highest medians for magnesium were found for the oldest women, while the lowest values were observed for the third age group. The control group displayed the highest median for the first age group, while the lowest magnesium concentration was observed in the pubic hair of the oldest women. The differences in magnesium concentrations in particular age groups were not statistically significant (Kruskal–Wallis one-way analysis of variance, $p>0.05$). In the experimental group, the highest zinc concentration occurred in the pubic hair of the oldest women, while the lowest was found for the youngest women. Zinc concentration in the hair of the women from the control group was the highest for the youngest women, i.e. below 29 years of age, the lowest having been found in the group of the oldest women. Like chromium, no significant statistical differences were found for zinc concentrations in particular age groups (Kruskal–Wallis one-way analysis of variance, $p>0.05$).

We also analysed the correlation between the concentrations of elements in hair and BMI (Fig. 1). The correlation coefficients for chromium and BMI pointed to a significant, proportional dependence between both parameters described by the coefficient $r=0.23$ ($p=0.03$). A similar result was obtained while analysing the dependence between magnesium concentration and BMI ($r=0.33$, $p=0.00$). On the other hand, zinc concentration in the female pubic hair

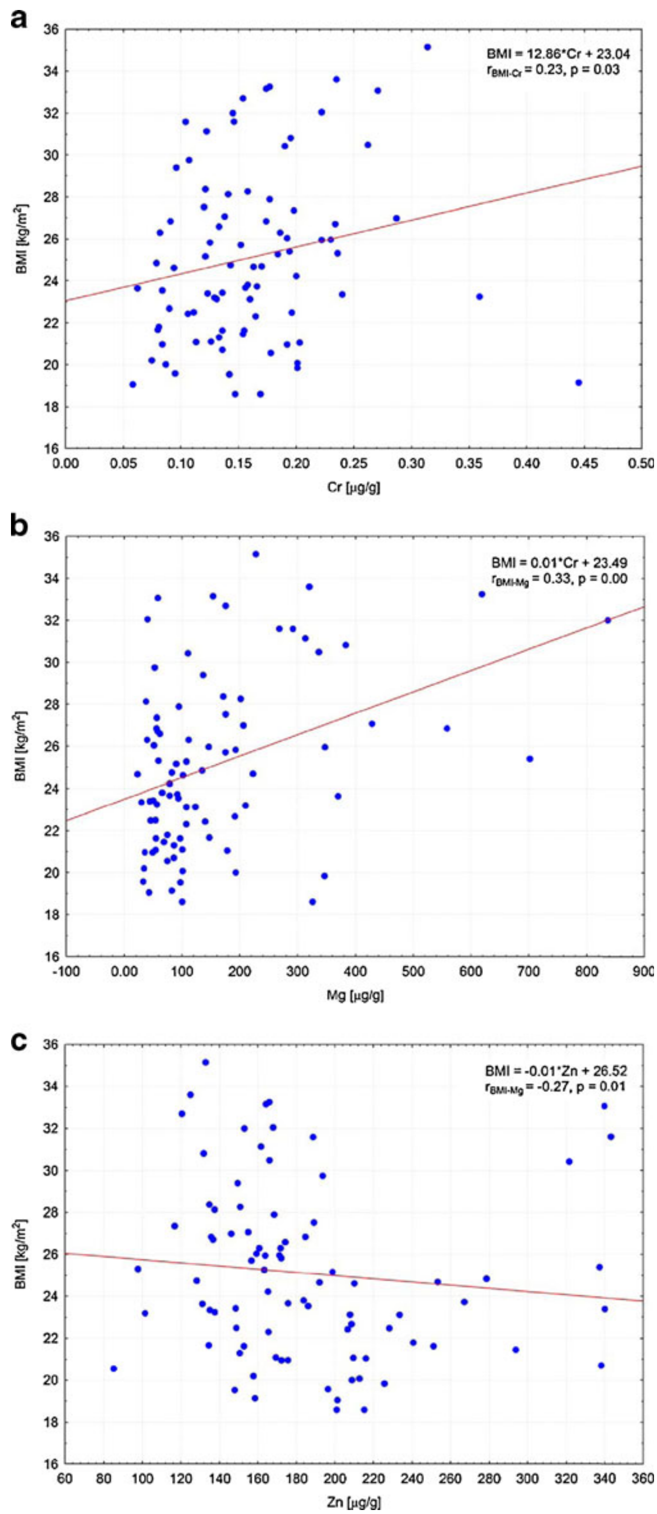

Fig. 1 Correlation between chromium (**a**), magnesium (**b**) and zinc (**c**) concentration in female pubic hair and BMI

decreased with increasing BMI, which was described by the correlation coefficient $r=-0.27$ ($p=0.01$).

The Zn/Mg and Cr/Mg quantitative ratios calculated were significantly different for the experimental and control

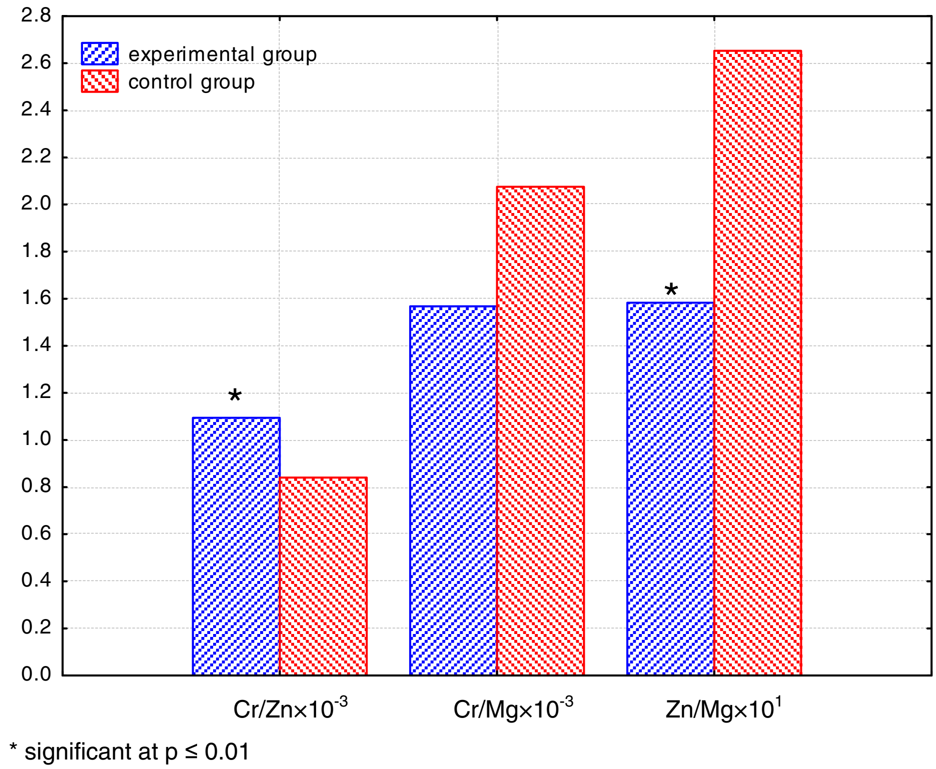

Fig. 2 Metals ratio in the pubic hair of women in the experimental and control groups. *Asterisks* indicate significance at $p \leq 0.01$

groups (Fig. 2). The mean Zn/Mg ratio for the control group was 2.65, being smaller for the experimental group—1.58. A particularly low Zn/Mg ratio was observed for the hair of the obese women. As to the Cr/Mg quantitative ratio, the values were as follows: 1.13×10^{-3} in the experimental group and 1.56×10^{-3} in the control group, and, like the Zn/Mg ratio, was particularly low for the obese women—0.76×10^{-3}. The Cr/Zn ratios reached 0.74×10^{-3} for the obese and overweight women, and 1.05×10^{-3} for the others and did not differ statistically.

Discussion

Recently, hair has become a desirable material for doing research. It certainly results from easy, non-invasive sample collection, long storage time and lack of complications after the collection. Moreover, since chemical elements are the permanent constituents of hair structure throughout its growth time, their concentrations in hair reflect their long-term levels in the organism, which enables the assessment of the mineral balance over a much longer period than the analysis of biological samples, such as blood or urine [12, 13]. Another advantage of hair analysis is the fact that the obtained results are not the effect of any homeostatic mechanisms, unlike the assays carried out on blood samples. Additionally, the concentrations of elements in hair are much higher than in other materials, which improves assays [13]. Hair has been successfully used in forensic medicine, toxicology (assessment of acute metal poisoning) and monitoring of environmental pollution [15–19]. Nevertheless, some researchers are sceptical about hair analysis for diagnosing nutrition state because of individual differences in age, gender, race, hair colour and environmental pollutants [17, 18]. Most authors, however, are very positive about hair as research material [12, 13, 20–22]. It is used, e.g. to analyse different health disorders [7, 23–27]. Because hair offers many advantages in research, this study was based on the elemental analysis of hair and was aimed at assaying the concentrations of chromium, zinc and magnesium, and finding correlations between those concentrations and the incidence of female overweight and obesity.

The hair samples were taken from 85 women living in the Podkarpackie Voivodeship, a relatively clean region, so the effect of environmental factors on the results seems to be negligible. The median chromium concentration in the pubic hair of the women from the experimental group was much higher than in the control group. The study also found a statistically significant correlation between chromium concentration in hair and BMI, indicating an increase in chromium level with increasing BMI. An analogous, positive correlation between chromium concentration and BMI has been found by other authors [17, 28]. On the other hand, Campbell [29] claims that chromium concentration in hair decreases when following a diet rich in white rice, white sugar or white bread, because they contain low levels of chromium (which is essential for energy production from carbohydrates) and therefore the metabolism uses chromium deposited in the organs. In that case, a chromium decrease in hair is proportional to obesity class. By contrast, Shin et al. [30] did not spot any correlation between chromium levels and obesity class. However, it is a well-known fact that chromium is involved in increasing lipolysis, thus reducing fat tissue in obese people. Therefore, it is advisable to carry out research on a larger number of people to find out explicitly if there is a difference in chromium concentrations in the hair of overweight and obese people.

The hair in the experimental group appeared to have a lower zinc concentration than in the control group and was decreasing proportionally to increasing BMI. A comparison of the results to those found by other authors points to a similar correlation. Wang et al. [25] tested female adults in Taiwan and found that the mean zinc concentration in hair was 181.3±91.2 μg/g in the experimental group (overweight and obese women) and 216±121.7 μg/g in the control group. Similarly, Skalnay and Demidov [24] showed that zinc concentration in the hair of obese women was markedly lower (170.2 μg/g) than in the control group (186.1 μg/g).

Magnesium concentration in the pubic hair of both overweight and obese women was considerably higher than in the hair of women whose BMI was within limits. The increase in magnesium concentration in the hair with increasing BMI was also indicated by another significant correlation coefficient r=0.33. Obesity is often the effect of insulin insensitivity or glucose intolerance. A state like that may be manifested twofold by increased and decreased magnesium levels. Its level is normally indirectly proportional to its resources in the body, so a high magnesium concentration in hair may indicate its low concentration in the organism, which is observed in obesity. Similar results were obtained by Bae and Cho [28] who found elevated magnesium concentrations in the hair of people with higher BMI. However, other authors have not found such a correlation. This is caused by the fact that in obesity, a low amount of magnesium in the body can result in low magnesium concentrations in hair, which points to the low mineral balance. For example, Wang et al. [25] found that a group of obese people (BMI >35) had a mean magnesium concentration of 156.8±112.5 μg/g, people whose BMI ranged from 26 to 35 had a mean concentration of 241.5±164.8 μg/g, while the control group displayed 268.3±174.6 μg/g. Also Skalnaya and Demidov [24] revealed a decrease in Mg concentration in the hair of obese women (84.6 μg/g) compared to the control group (118.7 μg/g).

Zinc concentration in hair corresponds with its concentration in blood. Marreiro et al. [31] found that a group of obese people had a lower zinc concentration in erythrocytes than the control group. They assayed Zn concentrations in erythrocytes, which also (like hair) allow the assessment of nutrition state over a longer period of time, because their lifetime lasts approx. 120 days. Their results indicated zinc deficiency in obese people. A similar correlation was found by Perrone et al. [32].

Numerous studies proved the significant role zinc plays during the regulation of body mass and metabolism. Moreover, the effect of zinc on insulin activity was observed and therefore its deficiency is often linked with the development of insulin resistance [31]. This is confirmed by the fact that the fat tissue of overweight and obese people produces adipokines which, in turn, induce adipocytes to produce a lot of substances, including proinflammatory factors. This results in a chronic inflammatory condition which induces the expression of metallothionein and *zinc-regulated transporters, iron-regulated transporter-like protein* zinc transporters in hepatocytes. The proteins create favourable conditions for zinc accumulation in liver and adipocytes, which might result in a low zinc concentration in hair [10].

A number of authors emphasize that the evaluation of the mineral balance is affected not only by the concentrations of elements in hair but also determination of quantitative ratios that reflect the mutual proportions between elements [33–36]. In this work, the differences concerned zinc/magnesium and chromium/magnesium concentration ratios. The lowest Zn/Mg ratio was typical of the obese females, which was first of all connected with the markedly higher concentration of magnesium in that group of women. This high concentration in the pubic hair of obese women also affected the Cr/Mg ratio in hair. Like Zn/Mg ratio, it was the lowest for the obese women despite the fact that the group revealed a higher chromium concentration compared with the other groups. The results of our research confirm that obesity causes numerous metabolism disorders, related to elemental disorders as well, which can be analysed assaying their concentrations in hair.

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