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Original Article

Correlation of Calcium, Copper, Iron, Magnesium and Zinc Content in Hair with Basal Metabolic Rate and Bioelectrical Impedance in Adolescent Females

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Abstract

Objective: The aim of this study was to investigate the correlation of calcium, copper, iron, magnesium and zinc content in hair with basal metabolic rate (BMR), expressed as kilocalories per body weight in kilograms (kcal/kg), and bioelectrical impedance percentage (BI%) in adolescent females.
Materials and Methods: One hundred and forty-eight adolescent females with differing BMR and BI% were randomly enrolled, and the calcium, copper, iron, magnesium and zinc content of their hair were simultaneously measured by an atomic absorption spectrophotometer.
Results: Positive correlations of iron (β=0.3326; p<0.01) and zinc (β=0.3519; p<0.05) content in hair with BMR were found, while inverse correlations of zinc (β=–0.4885; p<0.01) and iron (β=–0.2582; p<0.05) content in hair with BI% were found.
Conclusion: Iron and zinc content in hair seems to have a close relationship with BMR and BI% in adolescent females. Exactly how iron and zinc affects BMR and BI% in these subjects is still unclear and warrants further study. [*Tzu Chi Med J* 2008;20(1):58–62]

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1. Introduction

There are many micronutritional elements (minerals and trace elements) in the human body that directly or indirectly participate in metabolism (including the basal metabolic rate [BMR]) and play key roles in modulating it. More than 25% of the enzymes in the body require micronutritional elements in order to be activated and to function properly in metabolism [1–3]. Of these micronutritional elements, calcium, copper, iron, magnesium and zinc have been identified as being most essential for adolescent metabolism [4–8]. Bioelectrical impedance (BI) analysis is a commonly used method of estimating body composition. It is familiar in the consumer market as a simple instrument for estimating body fat [9]. Put in simple terms, the higher the percentage of BI (BI%), the greater the percentage of fat in the human body.

Hematological analysis has been commonly used in the determination of calcium, copper, iron, magnesium and zinc status in the human body [10]. However, such analysis usually shows the status of calcium, copper, iron, magnesium and zinc found in blood on the day of testing or in the previous 2–3 days (short term). Therefore, it only reflects the condition according to a specific point of time and any underlying causes are not known. In our previous studies [11,12], it was concluded that either blood or hair could reflect the burden of arsenic, copper, iron, selenium and zinc levels in the human body. Hair analysis has a special advantage in that the contents of calcium, copper, iron, magnesium and zinc can be backtracked over longer periods of time (3–6 months or even longer), and are less affected by daily diet [11,12].

Furthermore, analysis of hair samples is easy and harmless for subjects. In this study therefore, hair analysis was the preferred method to examine the association of calcium, copper, iron, magnesium and zinc content with BMR (kcal/kg) and BI% in adolescent females. There are no other documented reports using hair sample analysis to study the correlation of calcium, copper, iron, magnesium and zinc content with BMR and BI% in adolescent females of the same age. Most reports [13–15] have emphasized the influence of macronutrition in human obesity, but the roles of the micronutritional elements of calcium, copper, iron, magnesium and zinc have seldom been studied. Thus, our study may be helpful in studying the prevalence of adolescent female obesity from the micronutritional element perspective. Our study may reveal a new outlook in the prevalence of adolescent female obesity on the basis of an overall analysis of micronutritional elements in the hair.

2. Materials and methods

2.1. *Reagents*

Reagents used for the digestion procedures were purchased from E. Merck (Frankfurt, Germany). Standard solutions (1000 ppm in H_2O) of calcium, copper, iron, magnesium and zinc were obtained from the same company.

2.2. *Apparatus*

The containers were made of inert materials such as quartz, Teflon, and polypropylene. All containers were first immersed in 8 N HNO_3 for 24 hours, rinsed under de-ionized distilled water, and dried at ambient temperatures before use.

2.3. *Hair sampling and cleaning*

It is necessary to consider differences in hair samples. Calcium, copper, iron, magnesium and zinc concentrations are affected by sex and gender [11,12]. Stringent screening and quality control are required in collecting samples so that the accuracy of the analysis is not affected. Hair samples for this study were randomly selected from junior college students of nursing in northern Taiwan. Their lifestyle and eating habits had been pre-screened to be as similar as possible. None of the students had any record of disease.

Hair samples were collected from the nape section of each adolescent female. One hundred and forty-eight adolescent females, ranging from 15 to 19 years old, provided hair samples. Hair samples were cut near the scalp area with thin-blade stainless steel scissors. The length of hair samples ranged from 1.0 to 3.0 cm. Hair samples were accurately weighed to 1.000 ± 0.200 g [11,12].

Hair samples were then placed inside polyethylene bags and stored in an environment with controlled temperature (25°C) and humidity (65% relative humidity). Samples were twice immersed in a 65-mL mixture of normal hexane, ethyl alcohol, and acetone (4:2:1 v/v). Each immersion lasted 1.5 hours. Then, the samples were rinsed with de-ionized distilled water four times and immersed in 65 mL of acetone for 15 minutes. The samples were given a final rinse with de-ionized distilled water three times, filtered with paper dried at ambient temperatures, and prepared for the digestion procedure [11,12].

2.4. *Hair digestion*

Each hair sample was weighed (0.200 ± 0.100 g) and then placed inside a 250-mL microwave digester vessel. Ten milliliters of nitric acid was added and then the sample was heated in a microwave CEM-MD2000 microwave digester (CEM Corp., Matthews, NC, USA) using less than 30% power for 5 minutes. Then, 10 mL of de-ionized distilled water was added, followed by heating at 40% power for 25 minutes and 0% power heating for 10 minutes. Finally, hydrogen dioxide (H_2O_2) 2 mL was added, followed by 65% power heating for 5 minutes. After the heating procedures, the vessels were taken out under normal pressure and temperature [11,12]. All digested solutions were diluted to specific volumes with de-ionized distilled water for atomic absorption spectrophotometer determination.

2.5. *BMR and BI% analysis*

The BMR (expressed as kilocalories per body weight in kilograms, kcal/kg) and BI% were recorded from the same 148 adolescent females who had contributed the hair samples. This was done using a bioelectrical impedance analyzer (Olympia 3.5 body composition analyzer; Jawon Medical Co. Ltd., Seoul, Korea). BMR analysis was used to estimate the BMR in kcal/kg for different sexes and body heights based on the biological characteristics of body weight. The BMR is proportional to body weight and decreases with sex and body height, so BMR (kcal/kg) decreases according to increases in sex and body height. BI% analysis was used to estimate body composition using differences in conductivity based on the biological characteristics of tissue. Conductivity is proportional to water and electrolytes and decreases when the shape of the cell is closer to a round form. Adipose tissue is composed of round shaped cells and contains relatively less water than other tissues such as muscle, so conductivity decreases with increases in body fat.

The hair samples were collected and the BMR (kcal/kg) and BI% of the subjects were recorded at the same time. The whole sampling process was under the supervision of Superintendent Lee B.T., Director of the Student Activity Section, Mackay College of Medical Management and Nursing. All subjects provided informed consent as approved by the college's medical ethics committee.

2.6. *Atomic absorption spectrophotometer analysis*

A flame atomic absorption spectrophotometer (model Z-8200; Hitachi Corp., Tokyo, Japan) was used for determining calcium, copper, iron, magnesium and zinc levels [11,12]. The detailed instrument detection limits for calcium, copper, iron, magnesium and zinc are shown in Table 1.

2.7. *Accuracy and precision*

External standard methods were used for the quantitative determination of metal elements in hair. A series of standard solutions containing the following concentrations of calcium, copper, iron, magnesium and zinc ions were prepared using de-ionized distilled water (18 MΩ) and stock solutions (1000 ppm): 0.00, 0.10, 0.20, 0.40, 1.00, 2.00 and 4.00 μg/mL. To obtain accurate quantitative data, the regression coefficient of the standard calibration curve for each element was made greater than 0.9998.

Standard human hair samples (National Institute for Environmental Studies, Certified Reference Material, No. 13 human hair, Tokyo, Japan) were purchased and used to determine the precision and accuracy of calcium, copper, iron, magnesium and zinc measurements. Our results showed that the overall average recovery for these five elements was greater than 97.7%. Table 1 shows the recovery rate of these elements. Coefficients of variation percentage (CV%) for the standard materials were used for precision comparison. According to Table 1, the CV% for all five elements was less than 4.5%. Therefore, we concluded that our method was applicable to the analysis of calcium, copper, iron, magnesium and zinc content in hair.

2.8. *Statistical analysis*

STATISTICA version 6.0 (StatSoft Inc., Tulsa, OK, USA) was used to compute the statistical data. The data were analyzed by BMR (kcal/kg) and by BI% versus hair concentrations of calcium, copper, iron, magnesium and zinc in adolescent females. In multivariate regression (r), a p value of less than 0.05 was considered statistically significant. Values were expressed as mean ± standard deviation. The correlation coefficient (r) between two variables was computed in least squares regression equations.

3. Results

3.1. *Means and distributions of BMR and BI%*

As shown in Table 2, we found that BMR and BI% in adolescent females had a normal distribution.

Table 1 — Recoveries and coefficient variations of copper, iron, magnesium, zinc and calcium from standard human hair samples*†

	Certified value (μg/g)	CV%	Analyzed value (μg/g)	Recovery %	CV%	IDL (μg/g)
Copper	15.3±1.3	8.4	15.1±0.61	98.6	4.0	0.003
Iron‡	140		136.8±5.86	97.7	4.2	0.02
Magnesium‡	160		156.8±4.86	98.0	3.1	0.0002
Zinc	172±11	6.3	169.9±7.64	98.7	4.4	0.0015
Calcium‡	820		810.1±36.45	98.7	4.5	0.002

*Standard human hair samples were certified reference material purchased from the National Institute for Environmental Studies, Tokyo, Japan; †each value is the mean±standard deviation of three runs; ‡non-certified concentration of the constituent elements. CV% = coefficient of variation; IDL = instrument detection limit.

Table 2 — Distributions of basal metabolic rate (BMR) and bioelectrical impedance percentage (BI%) in adolescent females (n=148)*

	Mean±SD	Range
BMR (kcal/kg)	23.64±2.39	16.64–27.93
BI%	25.32±5.91	13.50–39.20

*Each value is the mean±standard deviation (SD) of three runs.

The mean contents of calcium, copper, iron, magnesium and zinc and their distribution of the BMR and BI% were 23.64±2.39 kcal/kg (range, 16.64–27.93 kcal/kg) and 25.32±5.91% (range, 13.50–39.20%).

3.2. *Correlation of BMR with body weight and BI%*

We found that in adolescent females, body weight had an inverse relationship with BMR (r=–0.8857; $p<0.0001$). Put in simple terms, the lower the BMR, the higher the body weight.

We also observed that in adolescent females, BMR had an inverse relationship with BI% (r=–0.7310; $p<0.0001$). In other words, the greater the BMR, the lower the BI%.

3.3. *Correlations of micronutritional elements with BMR by multivariate regression*

The R^2 value of multivariate regression between BMR and the hair content of calcium, copper, iron, magnesium and zinc was 0.3110 ($p<0.001$). Table 3 shows that the hair content of copper (β=0.1295; $p>0.05$), iron (β=0.3326; $p<0.01$), magnesium (β=0.0619; $p>0.05$) and zinc (β=0.3519; $p<0.05$) showed a positive correlation with BMR. In contrast, an inverse correlation of calcium content with BMR (β=–0.1037; $p>0.05$) was found in adolescent female hair. Iron was the most significantly related to BMR (β=0.3326; $p<0.01$).

3.4. *Correlations of micronutritional elements with BI% by multivariate regression*

The R^2 value of multivariate regression between BI% and the hair content of calcium, copper, iron, magnesium and zinc was 0.2810 ($p<0.001$). Table 3 shows that the hair content of calcium (β=0.1818; $p>0.05$) and magnesium (β=0.0170; $p>0.05$) show a positive correlation with BI%. In contrast, an inverse correlation of copper (β=–0.1312; $p>0.05$), iron (β=–0.2582; $p<0.05$) and zinc (β=–0.4885; $p<0.01$) content with BI% was found in adolescent female hair. Zinc was the most significantly related to BI% (β=–0.4885; $p<0.01$).

3.5. *Mean content and distribution of micronutritional elements in adolescent female hair*

The mean content and distributions of calcium, copper, iron, magnesium and zinc in adolescent female hair are shown in Table 3.

4. Discussion

It has been reported that the calcium content within a body is directly related to the decomposition of the fatty acid within the brown fat cells in subcutaneous tissues [16–18]. In other words, higher calcium content can decrease BI% by evaluating BMR. But in this study, we did not find the BMR elevating with increasing calcium content in adolescent female hair. On the contrary, we demonstrated that BI% is elevated by increased calcium content in adolescent female hair. Therefore, our results disagree with the finding of Zemel [18] who found that a higher calcium content in the body suppresses the formation of new fat and catalyzes the decomposition of existing fat.

The most important finding in this work is that adolescent female BI% decreases with higher iron content in hair (β=–0.2582; $p<0.05$). Clouet et al [19] pointed out that iron may have a direct impact on fatty acid oxidation and ATP production in the mitochondria for the body's metabolism to break down fatty acids and reduce BI%. Our findings agree with those of Clouet et al [19]. Nevertheless, our results further showed that BMR in adolescent females significantly increases with the iron content in their hair (β=0.3326; $p<0.01$). Since iron can play an important role in fatty acid metabolism to generate ATP in the mitochondria, we conclude that iron probably elevates adolescent female BMR and activates fatty acid metabolism (reduces BI%) in adolescent females.

Finally, we demonstrated that higher zinc content reduces BI% (β=–0.4885; $p<0.01$). Our findings agree with those of Anetor et al [20] who reported that zinc plays crucial roles in the regulation of carbohydrate and lipid metabolism in the human body. Furthermore, our results also showed that adolescent female BMR significantly increases with the zinc content in their hair (β=0.3519; $p<0.05$). A possible explanation is that zinc can enhance adolescent female BMR. We therefore conclude that zinc probably elevates BMR and activates fatty acid metabolism (reduces BI%) in adolescent females.

Table 3 — Beta values for hair content of different micronutritional elements versus basal metabolic rate (BMR) and bioelectrical impedance percentage (BI%) in adolescent females by multivariate regression ($n = 148$)

Micronutritional element	BMR (kcal/kg)		BI%		Mean content (μg/g)/kg	Range (μg/g)/kg
	β	p	β	p		
Calcium	−0.1037	>0.05	0.1818	>0.05	37.03±20.09	7.05–134.7
Copper	0.1295	>0.05	−0.1312	>0.05	0.28±0.25	0.05–1.61
Iron	0.3326	<0.01	−0.2582	<0.05	1.12±1.54	0.09–9.11
Magnesium	0.0619	>0.05	0.0170	>0.05	5.22±3.71	0.25–21.34
Zinc	0.3519	<0.05	−0.4885	<0.01	5.65±3.54	0.86–26.2

Basal metabolic rate expressed as calories per body weight in kilograms. (μg/g)/kg = mean content of micronutritional element in hair per gram per body weight in kilograms. The R^2 value of regression for BMR and BI% were 0.3110 ($p<0.001$) and 0.2810 ($p<0.001$), respectively.

In conclusion, since we know that an increase in the BMR is an important threshold for breaking down body fat [20], we believe that elevated iron and zinc content not only increases BMR but also decreases BI% in adolescent females. Our study may be able to serve as a new method in the study of the prevalence of adolescent female obesity on the basis of an overall analysis of hair micronutritional elements. However, exactly how iron and zinc affect BMR and modulate BI% in the human body is still unclear. Therefore, a larger-scale detailed study of how iron and zinc affect BMR and BI% in the human body is warranted.

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