Concentrations of Calcium, Copper, Iron, Magnesium, and Zinc in Young Female Hair with Different Body Mass Indexes in Taiwan

Chin-Thin Wang,*[, a] Wei-Tun Chang,[b] Lin-Her Jeng,[a] Po-En Liu,[c] and Li-Yun Liu[d]

[a]Department of Holistic Education Center St. John's and St. Mary's Institute of Technology, 499, Section 4, Tam-King Road, Tamsui, Taipei, Taiwan, Republic of China, [b]Department of Forensic Science, Central Police University, 56, Shu-Jen Road, Kueishan Taoyuan, Taiwan, Republic of China, [c]Mackay Junior College of Medical Management and Nursing, 92 Sheng-Ching Road, Taipei, Taiwan, Republic of China, and [d]Department of Food Science, Nutrition, and Nutraceutical Biotechnology, Shin Chien University, 70, Ta-Chi Street, Taipei, Taiwan, Republic of China

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An investigation of the metal concentrations of calcium, copper, iron, magnesium, and zinc in three groups of young female hair (n = 180), from women aged between 15 to 19 years, with different body mass indexes (BMI) of BMI < 18, BMI between 18 and 23, and BMI > 24 was performed using atomic absorption spectroscopy (AAS). The hair samples were washed with organic solvent (normal hexane : ethyl alcohol : acetone, v/v, 4 : 2 : 1) to remove the external contents and then digested with a microwave digester. The hair samples were analyzed using a flame atomic absorption spectrophotometer. The BMI < 18 group (n = 45) had the highest concentrations of calcium, copper, iron, magnesium and zinc while the BMI between 18 and 23 group (n = 75) had the second highest concentrations. The BMI > 24 group (n = 60) had the lowest concentrations. Further -more, when we compared the concentrations of calcium, copper, iron, magnesium, and zinc between the groups with BMI < 18 and BMI > 24, there are significant differences ($p < 0.05$) in the calcium, copper, magnesium, and zinc concentrations between these two groups. The difference was especially significant ($p < 0.01$) in iron concentration.

*To whom correspondence should be addressed: Department of Holistic Education Center St. John's and St. Mary's Institute of Technology, 499, Section 4, Tam-King Road, Tamsui, Taipei, Taiwan, Republic of China. Tel.: +886-2-28740682; Fax: +886-2-28748998; E-mail: wang6860@mail.sjsmit.edu.tw

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INTRODUCTION

There are many trace elements in the body, which directly or indirectly participate in metabolism and play key roles in modulating it. Approximately 25% of the enzymes in the human body require metals to be activated and function properly in metabolism.[1–3) Out of these metals calcium, copper, iron, magnesium, and zinc are the most crucial cations that required.[4)]

Calcium is one of the main components of bones and teeth and plays a part in nerve, muscle, and bone metabolism. It plays a vital role in maintaining the function of the heart, kidney, and platelets and in protecting the blood vessels.[4)] Furthermore, calcium helps the body to break down fat and burn carbohydrates.[5–7)] Copper cations are also required as cofactors for many enzymes such as cytochrome oxidase, the oxidizing enzyme, in the mitochondria. Cytochrome oxidase requires activation by copper cation to conduct electron transfer. Therefore, like iron, copper may also have an indirect impact on the fatty acid oxidation and adenosine 5′-triphospate (ATP) production in mitochondria. Copper is extremely critical in the body's oxygen delivery process. Without copper transferring iron to the proper position, iron will not be able to carry out its normal functions.[8)]

Iron cations are crucial for the delivery of oxygen within the body through interactions with hemoglobin and myoglobin. In addition to delivering oxygen, iron is also the main component of cytochrome C, which is responsible for electron transfer within mitochondria. Therefore in a high iron concentration may have an indirect impact on fatty acid oxidation and ATP production in mitochondria.[4,9,10)] Magnesium can help the bones grow, maintain stable metabolism, keep the blood vessels flexible, prevent cardiovascular disease and edema, and provide an antagonist function with calcium.[4,11,12)] Zinc cations are used as cofactors for many enzymes in the liver, for example, fructose-1, 6-bisphosphatase, alkaline phosphatase, and ethyl alcohol dehydrogenase.

Since these five elements are so crucial in the formation, break down, and metabolism of body fat, it is worth investigating them further.[4,12–14)] Martino *et al.*[15)] conducted studies on the concentration of zinc in the serum of obese subjects. They found by comparing to the body mass index (BMI) in different groups that the concentration of zinc in blood samples was significantly lower in the obese. Lin *et al.*[16] obtained similar results using atomic absorption spectrometry to analyze zinc concentrations in blood samples of overweight volunteers. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) is a highly sensitive method that uses argon plasma as the light source. ICP-AES is capable of detecting parts-per-billion (ppb) concentrations of trace elements and can detect multiple elements at the same time. Tsai *et al.*[17] used this equipment to analyze the concentration of metal in blood samples of Taiwanese residents. There is only limited research on the analysis of hair samples from young women (age from 15 to 19 years) with the different BMIs to determine calcium, copper, iron, magnesium, and zinc concentrations. Therefore our research provides a link between the range of the calcium, copper, iron, magnesium, and zinc concentrations and BMI. Since these five metal elements play such vital roles in metabolism, it is worth studying the relation between these five elements not only with obesity but also with health.

MATERIALS AND METHODS

Materials —— Reagents used for digestion procedures and deionized distilled water (18 MΩ) were purchased from E. Merck. Standard solutions (1000 ppm in H_2O) of calcium, copper, iron, magnesium, and zinc were obtained from the same company. Standard human hair sample NIES No. 5 (National Institute for Environmental Studies No. 5 human hair, Japan) was purchased and used as the basis for the determination of precision and accuracy. 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 hr, rinsed under deionized distilled water, and dried at ambient temperature before use.

Hairs Sampling and Cleaning[18,19] —— Hair samples for this research were all provided by students in residence at a junior college of nursing (Taipei). Their lifestyles and eating habits were all similar. Hair samples were collected from the nape section of each person. One hundred and eighty young females who provided hair samples ranged from 15 to 19 years of age. 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.

Hair samples were then placed inside polyethylene bags and stored in a controlled environment of temperature (25°C) and humidity (65% relative humidity). The procedure for sample cleaning was described elsewhere.[18,19] Samples were immersed in a 65 ml mixture of normal-hexane, ethyl alcohol, and acetone (4 : 2 : 1, v/v) two times. Each immersion lasted 1.5 hr. Then the samples were rinsed under deionized distilled water four times and immersed in acetone 65 ml for 15 min. The samples were given a final rinse under deionized distilled water three times, filtered with paper dried at ambient temperature, and prepared for the digestion procedure.

Hair Digestion[18,19] —— A hair sample was weighed (0.200 ± 0.100 g) and then placed inside a 250 ml microwave digester vessel. Nitric acid 10 ml was added and then heated in a microwave (CEM-MD-2000 microwave digester, U.S.A.) using less than 30% power for 5 min. Then 10 ml of deionized distilled water was added followed by 40% power heating for 25 min. and 0% power heating for 10 min. Finally, hydrogen dioxide (H_2O_2) 2 ml was added, followed by 65% power heating for 5 min. After the heating procedures, vessels were taken out under normal pressure and temperature. All digested solutions were diluted to specific volumes with deionized distilled water for atomic absorption spectroscopy (AAS) determination.

AAS Analysis —— With some minor variations in AAS conditions, the instrumentation and procedures (Hitachi Z-8200, Japan, Atomic Absorption Spectrophotometer coupled with Flame Atomizer) were the same as used in our earlier study.[18,19] The external standard method was 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 was prepared using deionized distilled water 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.

Accuracy and Precision —— The NIES No. 5 human hair Japan recovery is used for comparing the accuracy of calcium, copper, iron, magnesium, and zinc measurements. Our results showed that the overall average recovery for these five elements is greater than 98.0%. Table 1 shows the recovery rate

Table 1. Recoveries and Coefficients of Variation of Copper, Iron, Magnesium, Zinc, and Calcium in the Standard Material of NIES No. 5. Human Hair Japan

	Certified value (μg/g)	CV%	Analized value (μg/g)	Recovery%	CV%
Copper	0.72 ± 0.04	5.0	0.71 ± 0.04	99.8	2.8
Iron	2.55 ± 0.10	3.9	2.52 ± 0.11	100.2	2.1
Magnesium	20.0 ± 0.4	2.0	19.7 ± 0.6	98.0	4.2
Zinc	0.89 ± 0.06	6.7	0.87 ± 0.06	99.5	2.5
Calcium[a)]	0.09 mg/ml		0.091 ± 0.002	101.3	5.0

Each value is the mean ± S.D. of three runs. *a*) Noncertified concentration of the constituent elements. NIES, National Institute for Environmental Studies.

Table 2. Comparison of Calcium, Copper, Iron, Magnesium and Zinc Concentrations in Young Females with BMI < 18, BMI between 18 and 23, and BMI > 24

Elements (μg/g)	BMI < 18 (n = 45)	BMI 18–23 (n = 75)	BMI > 24 (n = 60)
Calicium	2207.5 ± 899.1[a)]	2126.2 ± 971.8[c)]	1665.5 ± 722.2
Range	4362.5–1200.2	5833.3–718.3	2848.6–451.2
Copper	17.69 ± 10.73[a)]	14.28 ± 7.99	12.06 ± 6.22
Range	44.5–8.4	42.7–4.2	32.9–5.2
Iron	52.17 ± 26.09[b)]	44.30 ± 26.05[c)]	28.47 ± 14.16
Range	127.1–29.5	127.5–16.8	67.2–3.2
Magnesium	285.9 ± 99.30[a)]	237.0 ± 148.1	202.9 ± 111.8
Range	452.2–130.9	789.4–102.4	389.9–55.9
Zin[c)]	296.6 ± 117.2[a)]	282.3 ± 96.22	238.2 ± 102.2
Range	600.0–162.4	529.5–116.3	531.4–55.1

Each value is the mean ± S.D. of three runs. *a*, *c*) $p < 0.05$, *b*) $p < 0.01$ significant differences from the BMI > 24.

of these elements. The recovery rates for calcium, iron, copper, zinc, and magnesium were 101.3, 100.2, 99.8, 99.5, and 98.0%, respectively. The coefficients of variation (CV%) for the standard materials were used for precision comparison. According to Table 1, the CV% for these five elements is less than 5.0% (calcium 5.0%, copper 2.8%, iron 2.1%, magnesium 4.2%, zinc 2.5%). Therefore we conclude that our method is applicable to the analysis of calcium, copper, iron, magnesium, and zinc concentrations in hair.

Statistical Analysis ——— The statistical graphic package (Statgraphic, U.S.A.) was used to complete the computation of various statistical data. If the p-value for the mean concentrations of calcium, copper, iron, magnesium, and zinc between any two groups was less than 0.05, the difference was considered significant. Values are expressed as mean ± S.D.

RESULTS

Comparison of Calcium, Copper, Iron, Magnesium, and Zinc Concentration in Young Female Hair with Different BMI

The calcium, copper, iron, magnesium, and zinc concentrations in hair samples from young women with different BMIs are shown in Table 2.

The calcium concentration was 2207.5 ± 899.1, 2126.2 ± 971.8, and 1665.5 ± 722.2 μg/g for BMI < 18, BMI between 18 and 23, and for BMI > 24, respectively. The range of calcium concentration was 4362.5–1200.2, 5833.3–718.3, and 2848.6–451.2 μg/g for BMI < 18, BMI between 18 and 23, and BMI > 24, respectively.

The copper concentration is 17.69 ± 10.73, 14.28 ± 7.99, and 12.06 ± 6.22 μg/g for BMI < 18, BMI between 18 and 23, and for BMI > 24, respectively. The range of copper concentration was 44.5–8.4, 42.7–4.2, and 32.9–5.2 μg/g for BMI < 18, BMI between 18 and 23, and BMI > 24, respectively.

The iron concentration was 52.17 ± 26.09, 44.30

± 26.05, and 28.47 ± 14.16 μg/g for BMI < 18, BMI between 18 and 23, and for BMI > 24, respectively. The range of iron concentration was 127.1–29.5, 127.5–16.8, and 67.2–3.2 μg/g for BMI < 18, BMI between 18 and 23, and BMI > 24, respectively.

The magnesium concentration was 285.9 ± 99.30, 237.0 ± 148.1, and 202.9 ± 111.8 μg/g for BMI < 18, BMI between 18 and 23, and for BMI > 24, respectively. The range of magnesium concentration is 452.2–130.9, 789.4–102.4, and 389.9–55.9 μg/g for BMI < 18, BMI between 18 and 23, and BMI > 24, respectively.

The zinc concentration was 296.6 ± 117.2, 282.3 ± 96.22, and 238.2 ± 102.2 μg/g for BMI < 18, BMI between 18 and 23, and BMI > 24, respectively. The range of zinc concentration was 600.0–162.4, 529.5–116.3 μg/g, and 531.4–55.1 μg/g for BMI < 18, BMI between 18 and 23, and BMI > 24, respectively.

The relationship between calcium, copper, iron, magnesium, and zinc concentrations in young female hair from donors with different BMIs are shown in Table 2. Calcium, copper, iron, magnesium, and zinc concentrations in the young female hair were inversely related to BMI. This means that the higher BMI, the lower the mean calcium, copper, iron, magnesium, and zinc concentrations among in hair.

Comparing the calcium, copper, iron, magnesium, and zinc concentrations between groups, only the groups with BMI < 18 and BMI between 18 and 23 did not show any significant difference ($p > 0.05$). When comparing other group combinations such as BMI between 18 and 23 and BMI > 24, only calcium concentration showed significant differences ($p < 0.05$). However, in the other group combinations with BMI < 18 and BMI > 24 the calcium, copper, magnesium, and zinc concentrations showed significant differences ($p < 0.05$), especially the iron concentration ($p < 0.01$).

DISSCUSION

Based on our current results, the change in the calcium concentration in young female hair may be closely associated with changes in BMI. In some other reports,[20,21] it was mentioned that "the calcium content within a body is directly related to the decomposition of the fatty acid within the brown fat cells in the subcutaneous tissues." "Higher calcium concentration in the body will suppress the formation of the new fat and catalyze the decomposition of the existing fat." In other words, the right amount of calcium might help people to lose weight.

Clouet *et al.*[22] pointed out that, like iron, copper may also have an indirect impact on fatty acid oxidation and ATP production in the mitochondria for the body's metabolism to break down the fatty acid and reduce fat. Bruner *et al.*,[23] Kretsh *et al.*[24] and Pinhas *et al.*[10] mentioned that adolescent girls with insufficient iron tend to have higher BMI and lower cognitive performance. In our previous study[25] we also found that the iron concentration in young female hair is inversely related to cognitive performance. From this point of view, insufficient iron may be affected not only the BMI but also cognitive performance in adolescent girls. Furthermore, our results agree with others[11–16] that people with higher BMIs have lower magnesium and zinc concentrations in their hair.

Based on these results, we recommend that growing young women ensure balanced nutrition to prevent becoming overweight. They should consume foods rich in calcium, copper, iron, magnesium, and zinc. A database of the concentrations of calcium, copper, iron, magnesium, and zinc in the hair of persons of different gender, age, profession, or working experience can be created to evaluate the relationship between BMI and these elements in each group. This database could be used to help weight control (prevent obesity) and even be used to identify unidentified bodies in forensic science.

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