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Original research

Assessment of diabetics by the quantification of essential elements and stable isotope ratios of carbon and nitrogen in scalp hair

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ABSTRACT

Aim: Assessment of diabetics was investigated by quantification of essential elements and $\delta^{13}C$ and $\delta^{15}N$ in the scalp hair, and the factors most related to the onset of diabetes identified by statistical analysis.
Methods: Essential elements and $\delta^{13}C$ and $\delta^{15}N$ values in the hair of diabetics and controls were quantified using ICP-MS and IR-MS.
Results: The Ca, Mg and Na concentrations in female controls were higher than those in male controls. Irrespective of gender, the Na and K concentrations in diabetics were higher than those in controls, while the Ca, Mg, Zn, Cu, Cr and Fe concentrations in diabetics were lower than those in controls. The $\delta^{13}C$ value was significantly lower in male diabetics that in male controls. The Ca, Mg, Cr and Fe concentrations in males were positively correlated to the $\delta^{13}C$ value and negatively correlated to the HbA1c value, whereas the Ca, Zn and Fe concentrations in females were positively correlated to the $\delta^{13}C$ value, with a negative correlation of Zn to HbA1c value.
Conclusion: Whether the subject was a diabetic or not could be determined on the basis of Zn, Cr and Na concentrations for males and Zn and Fe concentrations for females by the multiple logistic regression analysis.

1. Introduction

Human scalp hair offers an easy-to-use and conveniently obtainable biological sample; it can be noninvasively sampled, and records the dietary history and physiological conditions over a much longer timescale than does blood or urine (Petzke et al., 2010; Wołowiec et al., 2013). Quantification of essential and non-essential elements as well as stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in the scalp hair is currently used as diagnostic tool for a number of diseases (Wołowiec et al., 2013) as well as for the assessment of nutritional and metabolic status, food preferences and food supply (Petzke et al., 2010; Park et al., 2009, 2015; Endo and Haraguchi, 2010; Endo et al., 2017; Hayasaka et al., 2017).

According to the previous literature (Mooradian et al., 1994; Chen et al., 1995; Taneja et al., 1998; Skalnaya and Demidov, 2007; Kazi et al., 2008; Viktorínová et al., 2009; Wiernsperger and Rapin, 2010; Basaki et al., 2012; Hotta et al., 2018), diabetes has been linked to a compromised status of several elements such as Ca, Mg, Zn, Cu, Cr, Fe, Mn, V and Se. Among the elements reported to date, most studies reported lower concentrations of Mg, Zn and Cr in the scalp hair of diabetic subjects than control subjects (Hotta et al., 2018; Sanjeevi et al., 2018). The levels of toxic elements, such as Pb, Cd and As, in the scalp hair were reported to be higher in diabetic subjects than control subjects, and such increases are supposed to be associated with the onset of diabetes (Afridi et al., 2008).

Although the available data for Na and K in scalp hair are limited, the Na and K concentrations were shown to be higher in diabetic subjects than in control subjects (Skalnaya and Demidov, 2007). Like diabetes, Park et al. (2009) reported higher concentrations of Na and K and lower concentrations of Ca, Mg, Zn, Cu and Fe in the scalp hair of the metabolic syndrome subjects as compared with control subjects. Metabolic syndrome is a risk factor of diabetes. Further study of Na and K concentrations in the scalp hair of diabetics is therefore necessary.

The levels of some essential elements such as Ca and Mg in the scalp hair are known to be higher in Japanese (Kamakura, 1983) and Polish (Chojnacka et al., 2010) females than males, and the incidence of diabetes is higher in males than in females in Japan (Kuzuya, 1994) as well as in UK and other countries (Diabetes in the UK, 2010). These

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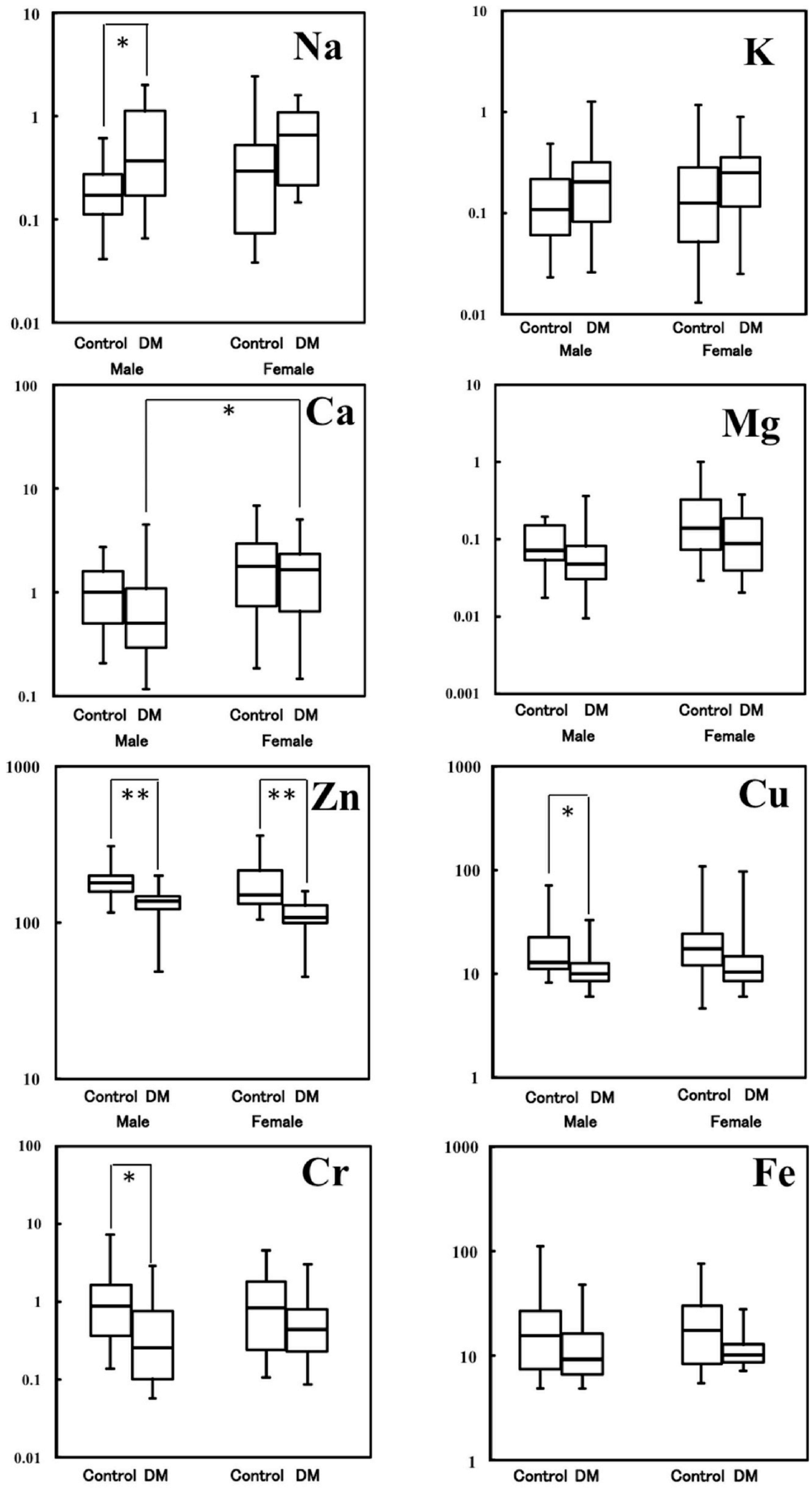

Fig. 1. Analytical results of Na, K, Ca, Mg, Zn, Cu, Cr and Fe concentrations in the scalp hair of control and diabetics (DM). The vertical axis is mg/g or μg/g, respectively. See Table 1. *$p < 0.05$, **$p < 0.01$.

differences led us to hypothesize that gender-related differences in essential elements in the scalp hair may be related to the gender-related difference in the incidence of diabetes. However, little information is currently available as only a few studies have examined the essential elements separately for male and female diabetics.

To our knowledge, there have been no reports on $\delta^{13}C$ and $\delta^{15}N$ values and essential elements in the same hair samples from diabetics. In the present study, we quantified 10 essential elements and 5 non-essential elements as well as $\delta^{13}C$ and $\delta^{15}N$ values in the scalp hair samples from diabetic and control subjects, and investigated the

Table 1
Analytical results for elements in the hair from the controls and diabetics.

	Control subjects	Diabetic subjects
Number[a]	Total n = 54	Total n = 42
	Male n = 23	Male n = 27
	Female n = 31	Female n = 15
Age (yr)	60.4 ± 15.6	66.8 ± 11.5
	Male 56.3 ± 14.3	Male 66.1 ± 13.2
	Female 63.4 ± 16.0	Female 68.0 ± 8.5
BMI (kg/m^2)		25.2 ± 4.7
	NA	Male 24.5 ± 3.8
		Female 26.5 ± 6.0
HbA1c (%)	5.2 ± 0.5 (n = 10)	9.0 ± 2.4
	Male 5.2 ± 0.4 (n = 4)	Male 9.0 ± 2.3
	Female 5.3 ± 0.5 (n = 10)	Female 9.0 ± 2.6
δ^{13}C (‰)	−19.3 ± 0.7	−19.7 ± 0.6**
	Male −19.0 ± 0.8^{A}	Male −19.6 ± 0.7^{B}
	Female −19.4 ± 0.6^{AB}	Female −19.8 ± 0.4^{B}
δ^{15}N (‰)	9.2 ± 0.5	9.3 ± 0.8
	Male 9.3 ± 0.4^{A}	Male 9.1 ± 0.8^{A}
	Female 9.1 ± 0.5^{A}	Female 9.6 ± 0.8^{A}
Na (mg/g)	0.36 ± 0.49	0.67 ± 0.57**
	Male 0.22 ± 0.15^{A}	Male 0.66 ± 0.61^{B}
	Female 0.47 ± 0.61^{AB}	Female 0.70 ± 0.53^{B}
K (mg/g)	0.19 ± 0.20	0.27 ± 0.26*
	Male 0.16 ± 0.13^{A}	Male 0.27 ± 0.29^{A}
	Female 0.21 ± 0.24^{A}	Female 0.27 ± 0.22^{A}
Ca (mg/g)	1.70 ± 1.37	1.20 ± 1.23**
	Male 1.12 ± 0.68^{AB}	Male 0.84 ± 0.92^{A}
	Female 2.13 ± 1.59^{B}	Female 1.84 ± 1.46^{B}
Mg (mg/g)	0.179 ± 0.218	0.097 ± 0.099**
	Male 0.094 ± 0.055^{AB}	Male 0.080 ± 0.087^{A}
	Female 0.243 ± 0.269^{B}	Female 0.128 ± 0.113^{AB}
Zn (μg/g)	184 ± 62	124 ± 36**
	Male 187 ± 44^{A}	Male 132 ± 37^{B}
	Female 183 ± 73^{A}	Female 110 ± 31^{B}
Cu (μg/g)	22.3 ± 18.9	13.7 ± 14.3**
	Male 21.0 ± 17.2 ^{A}	Male 12.1 ± 6.7^{B}
	Female 23.2 ± 20.2 ^{A}	Female 16.7 ± 22.3 ^{AB}
Cr (μg/g)	1.44 ± 1.57	0.69 ± 0.78**
	Male 1.42 ± 1.77 ^{A}	Male 0.62 ± 0.75 ^{B}
	Female 1.45 ± 1.43 ^{A}	Female 0.81 ± 0.85 ^{AB}
Fe (μg/g)	22.5 ± 20.7	12.9 ± 8.5**
	Male 23.0 ± 24.5^{A}	Male 13.3 ± 9.7 ^{A}
	Female 22.1 ± 17.8 ^{A}	Female 12.2 ± 6.1 ^{A}
Mn (μg/g)	0.95 ± 3.62	1.46 ± 7.09
	Male 0.57 ± 1.17 ^{A}	Male 0.43 ± 0.57 ^{A}
	Female 1.23 ± 4.69 ^{A}	Female 3.30 ± 11.9 ^{A}
V (μg/g)	0.033 ± 0.042	0.024 ± 0.031*
	Male 0.031 ± 0.024 ^{A}	Male 0.025 ± 0.038 ^{A}
	Female 0.035 ± 0.052 ^{A}	Female 0.022 ± 0.010 ^{A}
Se (μg/g)	0.71 ± 0.36	0.68 ± 0.38
	Male 0.87 ± 0.19 ^{A}	Male 0.69 ± 0.24 ^{AB}
	Female 0.59 ± 0.41 ^{B}	Female 0.67 ± 0.56 ^{B}
As (μg/g)	0.043 ± 0.033	0.076 ± 0.071
	Male 0.062 ± 0.033 ^{A}	Male 0.088 ± 0.071 ^{A}
	Female 0.029 ± 0.025^{B}	Female 0.054 ± 0.067 ^{AB}
Cd (μg/g)	0.016 ± 0.015	0.019 ± 0.027
	Male 0.012 ± 0.011 ^{A}	Male 0.019 ± 0.033 ^{A}
	Female 0.020 ± 0.017 ^{A}	Female 0.020 ± 0.014 ^{A}
Hg (μg/g)	1.85 ± 2.12	2.15 ± 1.60
	Male 2.50 ± 2.94 ^{A}	Male 2.12 ± 1.49 ^{AB}
	Female 1.37 ± 1.03 ^{B}	Female 2.19 ± 1.83 ^{AB}
Pb (μg/g)	0.593 ± 0.483	0.960 ± 1.988
	Male 0.632 ± 0.521 ^{A}	Male 0.795 ± 1.112 ^{A}
	Female 0.565 ± 0.459 ^{A}	Female 1.258 ± 3.021 ^{A}

Significantly different from Control subjects (*$p < 0.05$, **$p < 0.01$).
Different letters (A and B) indicate a significant difference among the four sites ($p < 0.05$, Turkey-Kramer).

[a] Numbers of Control and DM subjects except for HbA1c of Control subject.

correlations among the 15 elements and δ^{13}C and δ^{15}N values separately for males and females, the elements that contribute most to diabetes in males and females, and distinguished between subjects having diabetes or not using multivariate statistical analysis of those values.

2. Materials and methods

2.1. Ethics statement

This research project and associated procedures were approved by the Human Research Ethics Committee of the Graduate School of Pharmaceutical Sciences, Health Sciences University of Hokkaido (No.15P004), and the Nikko Memorial Hospital (No. 80). Written informed consent was obtained from hair donors prior to participation in this study.

2.2. Sampling of scalp hair

Scalp hair samples from 42 diabetic (27 males and 15 females) and 54 control subjects (23 males and 31 females) were collected during November 2009 and April 2016 as reported previously (Hotta et al., 2018). The hair samples were applied for the quantification of δ^{13}C and δ^{15}N and 15 elements.

2.3. Analyses of stable isotope ratios of carbon and nitrogen in scalp hair

The δ^{13}C and δ^{15}N values in the scalp hair samples (about 1 mg) after the removal of lipids using chloroform/methanol extraction were analyzed using a mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany) coupled with an elemental analyzer (EA1108, Fisons, Roano, Milan, Italy), as described previously (Hayasaka et al., 2017; Endo et al., 2015).

2.4. Analyses of elements in scalp hair

As reported previously (Hotta et al., 2018), the washed hair samples (about 20–25 mg) were digested by nitric acid at 75 °C for 2 h. After cooling to room temperature and adjusting the gravimetric volume, the resultant digestion was used for the analysis of elements. Fifteen elements, Na, K, Ca, Mg, Zn, Cu, Fe, Cr, Mn, Se, V, As, Cd, Hg and Pb, were simultaneously measured by inductively coupled plasma mass spectrometry (ICP-MS; Agilent-7700). The element concentrations were expressed as ng/g hair or μg/g hair.

The accuracy of our analysis was assessed using certified reference materials (CRMs) from NIES, Japan (Human hair No. 13), and from NCS, China (Human hair No. ZC81002b). Our analytical data for the 15 elements were in good agreement with the certified values. Recoveries of the elements from NIES No 13 and NCS No. Z81002b were 77–105% and 76–114%, respectively.

The analytical data for 12 elements in 38 diabetic and in all control subjects was previously reported (Hotta et al., 2018).

2.5. Statistical analyses

Statistical analyses of the data except for the 15 elements in the scalp hair were analyzed by Student's t-test or Scheffe's F-test using the Statcel 2 program (OMS, Japan). Statistical analyses of the 15 elements were conducted by Welch's t-test using SPSS statistics 25 (Illinois, USA) after logarithmic transformation, as the concentrations of those elements were log-normally distributed (Kamakura, 1983; Usuda et al., 2002). The data for elements shown Fig. 1 were expressed as box-and-whisker plots.

Principal component analysis of 8 essential elements was conducted to classify the elements that were related to the onset of diabetes and identify differences between male and female subjects using SPSS statistics 25.

Multiple logistic regression analysis was employed to determine which elements are most strongly related to diabetes, and to estimate whether the subject belonged to the control or diabetic group using

SPSS statistic 25.

3. Results

The diabetic subjects included 27 males (66.1 ± 13.2 years) and 15 females (68.0 ± 8.5 years) (Table 1). The BMI of males and females was 24.2 ± 3.8, and 26.5 ± 6.0 kg/m^2, respectively, and the HbA1c was 9.0 ± 2.3 and 9.0 ± 2.6%, respectively. The BMI of 8 males and 7 females exceeded the standard value of 25 kg/m^2 (maximum was 35.9 kg/m^2), and one female was below the standard value of 18.5 kg/m^2 (16.8 kg/m^2). The HbA1c of 2 males and 1 female exceeded 14%. No gender-related differences were observed among those characteristics.

Although the BMI of the control subjects was unknown, all appeared to be within the standard value (18.5–25 kg/m^2) based on their physical appearance. Available information for HbA1c was 5.2 ± 0.4% for 4 males and 5.3 ± 0.5% for 10 females.

The $\delta^{13}C$ value of the diabetic subjects (−19.7 ± 0.6‰, n = 42) was significantly lower than that of the controls (-19.3 ± 0.7‰, n = 54) (Table 1), with the $\delta^{13}C$ values of diabetic males (n = 27) and females (n = 15) being significantly and marginally lower than those of the control males (n = 23) and females (n = 31), respectively. On the other hand, $\delta^{15}N$ value of the female diabetics (9.6 ± 0.8‰, n = 15) was marginally higher than that of the male diabetics (9.1 ± 0.8‰, n = 27), and marginally higher than that of male and female controls (9.3 ± 0.4‰, n = 23 and 9.1 ± 0.5‰, n = 31, respectively).

The analytical results for the 15 elements in the scalp hair of the controls and diabetics are shown in Table 1, and the results for 8 elements are shown in Fig. 1.

Irrespective of gender (Table 1 and Fig. 1), the Na and K concentrations were higher in the diabetics than in the controls (a significant difference was found in the Na concentration for males, $p < 0.05$), while the concentrations of the other essential elements (Ca, Mg, Zn, Cu, Cr and Fe) were lower in the diabetics (significant differences were found in the Zn concentrations for males and females, and in the Cu and Cr concentrations for males, $p < 0.05$). The Na, Ca and Mg concentrations in female control and diabetic subjects were slightly higher than corresponding concentrations in the males, respectively.

The Se concentration in male controls was slightly but significantly higher than that in female controls ($p < 0.05$), although no gender-related difference was found in the diabetic subjects and no difference was found in the males and females between the control and diabetic subjects (Table 1).

The As and Hg concentrations in the controls were both significantly higher in males than in females ($p < 0.05$) (Table 1). No marked differences were found in the Pb and Cd concentrations between males and females or between the control and diabetic subjects. Further, no marked differences was found in the Mn and V concentrations between males and females or between the control and diabetic subjects.

Table 2 shows the correlation matrix among eight essential elements, HbA1c, and $\delta^{13}C$ and $\delta^{15}N$ values, calculated separately for the male and female groups comprised of the control and diabetic subjects. Logarithmic transformation of Na, K, Ca, Mg, Zn, Cu, Cr and Fe concentrations were chosen and applied for the calculations. In the case of the controls for whom HbA1c values were unknown, the values for male and female subjects were estimated to be 5.2 and 5.3%, respectively (Hotta et al., 2018).

In both the males and females, high correlations ($p < 0.01$) were found between the Na and K concentrations (γ = 0.543 and 0.718, respectively), the Ca and Mg concentrations (γ = 0.829 and 0.850, respectively) and the Cr and Fe concentrations (γ = 0.712 and 0.789, respectively). In addition, several moderate correlations ($p < 0.05$) were found in males (Ca-Cu, Ca-Cr, Mg-Zn, Mg-Cu and Mg-Cr) and in females (Ca-Na, Ca-Zn, Na-Mg and Mg-Zn).

The HbA1c of males was positively correlated to the Na and K concentrations (a significant difference was found for Na concentration), while it was negatively correlated to the Ca, Mg, Zn, Cu, Cr and Fe concentrations (significant differences were found for the Ca, Mg, Zn, Cu and Cr concentrations). On the other hand, the HbA1c of females was positively correlated to the Na and K concentrations ($p < 0.05$), but it was negatively correlated to the Zn concentration in Table 2 ($p < 0.05$) and the Cr and Fe concentrations ($p < 0.10$).

The $\delta^{13}C$ value of males was positively correlated to the Ca, Mg, Cr and Fe concentrations ($p < 0.05$), while that of females was positively correlated to the Ca, Zn and Fe concentrations ($p < 0.05$). The $\delta^{15}N$ value of males was correlated to the Cr concentration ($p < 0.05$), while no correlations were found for the females.

Logarithmic transformation of the concentrations of eight elements and $\delta^{13}C$ and $\delta^{15}N$ values was applied for principal component analysis followed by varimax rotation separately for males and females (Table 3), and investigated to determine which elements as well as $\delta^{13}C$ and $\delta^{15}N$ values were the most strongly related to the onset of diabetes. The combined 1st, 2nd, 3rd and 4th components (eigenvalues over 1) accounted for 82.7% of the total variance of males and 86.0% of that of females, respectively.

In males, Ca, Mg and Cu exclusively (eigenvector over 0.3) loaded on the 1st component, Cr and Fe exclusively loaded on the 2nd component, while Na and K exclusively loaded on the 3rd component, and Zn exclusively loaded on the 4th component. The scores for the 1st, 2nd 3rd and 4th components differed marginally or significantly between the control and diabetic subjects. Scores for the 1st and 2nd components were significantly correlated to the corresponding $\delta^{13}C$ value, and the score for the 2nd component was significantly correlated to the $\delta^{15}N$ value.

On the other hand, Ca, Mg and Zn in females exclusively loaded on the 1st component, while Cr and Fe exclusively loaded on the 3rd component, Na and K exclusively loaded on the 2nd component, and Cu exclusively loaded on the 4th component. Scores for the 1st and 2nd components for females differed significantly different between the control and diabetic subjects. The scores for the 1st and 3rd components for females were significantly and marginally correlated to the $\delta^{13}C$ value, respectively, while no correlations were found for the 2nd or 4th component scores. No correlations were found for the $\delta^{15}N$ value and each of the 1st, 2nd and 3rd component scores in females.

Multiple logistic regression analysis was applied to determine which factors (among the 8 elements, $\delta^{13}C$ and $\delta^{15}N$ values) are most strongly related to diabetes and to distinguish whether or not the subject had diabetes using SPSS statistic 25. The following formulae were derived: the formula for males included the logarithmic transfer values for the Cr, Na and Zn concentrations (Chi-squared test, $p < 0.01$), and that for females included the logarithmic transfer values for Fe and Zn concentrations (Chi-squared test, $p < 0.01$). If the p-value calculated from the score was bigger than 0.5, the subject was regarded as having diabetes whereas a smaller value indicated the subject was physically unimpaired.

$$\text{Males Score} = -3.465 \times [Cr] + 4.736 \times [Na] - 21.329 \times [Zn] + 95.097$$

$$\text{Females Score} = -4.459 \times [Fe] - 19.064 \times [Zn] + 115.244$$

$$p = 1/(1 + \exp(-1 \times \text{Score}))$$

Odds ratios for Zn in both formulae for males and female were almost zero, and the coefficient for Zn was much bigger than the coefficients for Cr and Na (males) and for Fe (females). The percentages of correct classification of the control and diabetic subjects among males were 87 and 89%, respectively, and those for control and diabetic subjects among females were 90 and 87%, respectively.

4. Discussion

The Ca, Mg, Zn, Cu, Cr and Fe concentrations in the scalp hair of

Table 2
Correlation matrix of eight elements and stable isotope ratios of carbon and nitrogen in males and females.

	Na	K	Ca	Mg	Zn	Cu	Cr	Fe	HbA1c	$\delta^{13}C$	$\delta^{15}N$
Males											
Na	1	**0.543**	−0.081	−0.103	−0.169	−0.177	0.003	0.102	**0.296**	−0.021	0.128
K	**0.543**	1	−0.174	−0.239	−0.170	−0.146	−0.209	−0.122	0.210	−0.084	−0.045
Ca	−0.081	−0.174	1	**0.829**	0.240	**0.596**	**0.299**	0.124	**−0.280**	**0.339**	0.070
Mg	−0.103	−0.239	**0.829**	1	**0.353**	**0.468**	**0.327**	0.134	**−0.301**	**0.315**	0.099
Zn	−0.169	−0.170	0.240	**0.353**	1	0.226	0.274	0.179	**−0.390**	0.113	−0.064
Cu	−0.177	−0.146	**0.596**	**0.468**	0.226	1	0.247	0.129	**−0.403**	0.214	0.223
Cr	0.003	−0.209	**0.299**	**0.327**	0.274	0.247	1	**0.712**	**−0.393**	**0.371**	**0.403**
Fe	0.102	−0.122	0.124	0.134	0.179	0.129	**0.712**	1	−0.228	**0.360**	0.245
HbA1c	**0.296**	0.210	**−0.280**	**−0.301**	**−0.390**	**−0.403**	**−0.393**	−0.228	1	**−0.369**	−0.142
$\delta^{13}C$	−0.021	−0.084	**0.339**	**0.315**	0.113	0.214	**0.371**	**0.360**	**−0.369**	1	**0.471**
$\delta^{15}N$	0.128	−0.045	0.070	0.099	−0.064	0.223	**0.403**	0.245	−0.142	**0.471**	1
Females											
Na	1	**0.718**	**0.304**	**0.405**	−0.230	−0.078	0.012	0.063	0.366	−0.127	0.138
K	**0.718**	1	0.148	0.178	−0.108	0.006	−0.074	0.031	**0.347**	0.046	−0.065
Ca	**0.304**	0.148	1	**0.850**	**0.388**	0.168	0.010	0.034	0.083	**0.299**	0.228
Mg	**0.405**	0.178	**0.850**	1	**0.426**	0.182	−0.053	−0.023	−0.048	−0.187	0.108
Zn	−0.230	−0.108	**0.388**	**0.426**	1	0.059	−0.176	−0.037	**−0.376**	**0.394**	−0.108
Cu	−0.078	0.006	0.168	0.182	0.059	1	0.215	0.145	−0.127	0.053	−0.084
Cr	0.012	−0.074	0.010	−0.053	−0.176	0.215	1	**0.789**	−0.226	0.196	−0.053
Fe	0.063	0.031	0.034	−0.023	−0.037	0.145	**0.789**	1	−0.256	**0.304**	−0.003
HbA1c	**0.366**	**0.347**	0.083	−0.048	**−0.376**	−0.127	−0.226	−0.256	1	−0.189	0.116
$\delta^{13}C$	−0.127	0.046	**0.299**	0.187	**0.394**	0.053	0.196	**0.304**	−0.189	1	−0.082
$\delta^{15}N$	0.138	−0.065	0.228	0.108	−0.108	−0.084	−0.053	−0.003	0.116	−0.082	1

Bold numbers indicate significant correlations ($p < 0.05$).

Table 3
Principal component analysis for 8 elements in the scalp hair of males and females.

		1st component	2nd component	3rd component	4th component
Males					
Eigenvalue		2.30	1.75	1.55	1.01
Contribution (%)		28.75	21.88	19.41	12.63
	Na	0.052	0.104	**0.583**	−0.024
	K	0.037	−0.102	**0.577**	0.133
	Ca	**0.456**	−0.057	0.070	−0.118
	Mg	**0.381**	−0.057	0.051	0.070
	Zn	−0.112	−0.066	0.060	**1.040**
	Cu	**0.366**	−0.034	−0.014	−0.147
	Cr	−0.012	**0.511**	−0.015	−0.028
	Fe	−0.099	**0.578**	0.020	−0.078
Component score	Control	0.28 ± 0.85	0.27 ± 1.07	−0.34 ± 0.71	0.46 ± 0.64
	DM	−0.24 ± 1.07#	−0.22 ± 0.89#	0.29 ± 1.13**	−0.39 ± 1.09**
Correlation between component score and $\delta^{13}C$	r	0.296*	0.351*	−0.015	0.019
Correlation between component score and $\delta^{15}N$	r	0.134	0.353*	0.051	−0.140
Females					
Eigenvalue		2.15	1.90	1.81	1.02
Contribution (%)		26.93	23.76	22.59	12.71
	Na	0.009	**0.487**	0.025	−0.079
	K	−0.068	**0.473**	−0.051	0.054
	Ca	**0.406**	0.041	0.045	0.002
	Mg	**0.401**	0.075	0.000	0.028
	Zn	**0.397**	−0.271	−0.012	−0.165
	Cu	−0.061	−0.004	−0.079	**0.998**
	Cr	−0.008	−0.023	**0.520**	0.000
	Fe	0.046	−0.012	**0.544**	−0.135
Component score	Control	0.25 ± 0.96	−0.26 ± 1.03	0.16 ± 1.10	0.12 ± 1.00
	DM	−0.52 ± 0.91*	0.54 ± 0.68**	−0.33 ± 0.68#	−0.26 ± 0.99
Correlation between component score and $\delta^{13}C$	r	0.357*	−0.129	0.266#	−0.035
Correlation between component score and $\delta^{15}N$	r	0.104	0.085	−0.004	−0.076

# $p < 0.10$, *$p < 0.05$, **$p < 0.01$.

diabetes sufferers were lower than the corresponding concentrations in the controls, irrespective of gender (Table 1 and Fig. 1). Many research groups have been reported decreases in some of these elements, with the decreases appearing to be related to the onset of diabetes, at least in part (Mooradian et al., 1994; Chen et al., 1995; Taneja et al., 1998; Skalnaya and Demidov, 2007; Kazi et al., 2008; Viktorínová et al., 2009; Wiernsperger and Rapin, 2010; Basaki et al., 2012; Hotta et al., 2018). On the other hand, the Na and K concentrations in the hair of diabetics were higher than the corresponding concentrations in the controls, irrespective of gender. Reflecting the increases in Na and K concentrations and decreases in Ca, Mg, Zn, Cu, Cr and Fe concentrations in the hair of diabetics, the Na concentration in males was positively correlated to the HbA1c value ($p < 0.05$) while the Ca, Mg, Zn, Cu and Cr concentrations were negatively correlated to HbA1c value ($p < 0.05$), whereas the Na and K concentrations in females were positively correlated to the HbA1c value ($p < 0.05$) while the Zn concentration was negatively correlated to the HbA1c value ($p < 0.05$) (Table 2). We are the first to report a positive correlation between the Na concentration in the scalp hair and the HbA1c value and a negative correlation between the HbA1c value and some essential elements. Although the available information on Na and K concentrations in the hair of diabetes is limited, Skalnaya and Demidov (2007) reported that the Na and K concentrations in the scalp hair of women were significantly higher in diabetic and obese subjects than in control subjects, while the Ca, Mg and Zn concentrations were significantly lower in the subjects with diabetes and obesity.

Strong correlations were found between the Na and K concentrations, the Ca and Mg concentrations, and the Cr and Fe concentrations in both males and females ($p < 0.01$) (Table 2). To our knowledge, the correlation between the Fe and Cr concentrations in the scalp hair has not yet been investigated, although the correlations and/or ratios of Na to K concentration and of Ca to Mg concentration have been well investigated. The role of the positive correlation between the Cr and Fe concentrations is currently unknown. In addition to those strong correlations, several moderate correlations ($p < 0.05$) were found among the Ca, Mg, Zn, Cu, Cr and Fe concentrations in males, and a few moderate correlations were found among those elements in females. According to a study analyzing 33 elements in scalp hair samples from Polish subjects (total number of male and female subjects was 83) (Chojnacka et al., 2010), no significant correlation was found between the concentrations of Na and essential elements (they did not measure the K concentration), and several moderate correlations were found among the Ca, Mg, Zn and Cu concentrations. However, they did not consider the correlations separately by gender.

The δ^{13}C values for the male and female diabetics were significantly and marginally lower than corresponding values for male and female controls, respectively (Table 1). The δ^{13}C value for males was negatively correlated to the HbA1c value ($p < 0.05$), while no correlation was found for the females (Table 2). The δ^{15}N value of female diabetics was marginally higher than that of male diabetics, and marginally higher than that of male and female control subjects. The Na and K concentrations in males and females were not correlated to the δ^{13}C value, while the Ca, Mg, Cr and Fe concentrations in males were significantly correlated to this value, and the Ca, Zn and Fe correlations in females were significantly correlated to this value (Table 2). We are the first to report significant and marginal decreases in δ^{13}C values in the scalp hair of diabetic males and females, respectively, the significant correlation between the δ^{13}C and HbA1c values in males, significant correlations between the δ^{13}C value and the Ca, Mg, Cr and Fe concentrations in males, and the higher δ^{15}N value in female diabetics than male diabetics ($p < 0.10$). In addition, we found that the Na, K and Ca concentrations in females tended to be higher than those in males among the Japanese control subjects (Fig. 1) which is in agreement with the previous report by Kamakura (1983). In this study, we found many gender-related differences in element concentrations and δ^{13}C values in the scalp hair of the control and diabetic subjects.

Principal component analysis of 8 elements in males and females led to classification into 4 groups: Ca and Mg loaded on the 1st component for males and females, Cr and Fe loaded on the 2nd component of males and on the 3rd component of females, and Na and K loaded on the 3rd component of males and on the 2nd component of females (Table 3). Cu was loaded with Ca and Mg (1st component) and Zn was loaded on the 4th component in males, while Zn was loaded with Ca and Mg (1st component) and Cu was loaded on the 4th component in females.

The scores for the 1st component in males (Ca, Mg and Cu) and in females (Ca, Mg and Zn) differed significantly between the control and diabetic subjects, and were significantly correlated with the δ^{13}C value, but not with the δ^{15}N value (Table 3). The scores for the 2nd component in males and the 3rd component in females (Cr and Fe) differed significantly between the control and diabetic subjects. This score (Cr and Fe) in males was significantly correlated with the δ^{13}C and δ^{15}N values, while the score in females tended to be correlated to the δ^{13}C values. The scores for the 3rd component in males and the 2nd component in females (Na and K) differed significantly between the control and diabetic subjects, but those scores were not correlated with the δ^{13}C or δ^{15}N values. Zn in males was exclusively loaded on the 4th component and differed significantly between the control and diabetic subjects, but showed no correlations with the δ^{13}C or δ^{15}N values. Thus, the principal component analysis of 8 elements (Table 3) was classified into 4 groups and the several correlations, such as between the HbA1c value and the concentrations of elements, between the δ^{13}C value and the concentrations of elements, and the gender-related differences, are summarized in Table 2.

Metabolic syndrome is known to be one of the risk factor for diabetes. Park et al. (2009) reported the essential elements in the scalp hair of Koreans with metabolic syndrome, and thereafter a different group reported the δ^{13}C and δ^{15}N values in the scalp hair of metabolic syndrome sufferers in Korea (Park et al., 2015). The Na and K concentrations were significantly higher in the metabolic syndrome than the control subjects, while the Ca, Mg, Zn and Cu concentrations were markedly lower in metabolic syndrome subjects (Park et al., 2009). On the other hand, the δ^{15}N value in the scalp hair of metabolic syndrome subjects in Korea was slightly but significantly higher than that in the control subjects (11.68 ± 0.92‰ vs. 11.53 ± 0.81‰, $p < 0.05$), while the δ^{13}C values were similar in both groups of subjects (−20.46 ± 0.92‰ vs. −20.37 ± 0.87‰) (Park et al., 2015). The changes in essential elements found in diabetes patients (Table 2) are similar to those found in the previous report on subjects with metabolic syndrome in Korea (Park et al., 2009), but the changes in δ^{13}C and δ^{15}N values in the diabetes patients (Table 1) do not agree with those in the metabolic syndrome subjects in Korea (Park et al., 2015). The Korean studies (Park et al., 2009, 2015) did not consider gender-related differences in the essential elements or in the δ^{13}C or δ^{15}N values.

The δ^{13}C and δ^{15}N values in the control subjects differed markedly between the present study of Japanese subjects (Table 1) and the previous study of Korean subjects (Park et al., 2009), and between the previous studies in Korea by Park et al. (2009) and by Endo et al. (2015) As the δ^{13}C and δ^{15}N values in the scalp hair are affected not only by disease but also food preferences and geographical factors (Endo and Haraguchi, 2010; Endo et al., 2015; Hülsemann et al., 2015; Kusaka et al., 2016), strict comparisons with a control group are necessary for drawing any definitive conclusions.

The Zn concentrations in males and females were significantly lower in diabetic subjects than in the control subjects (Table 1), and the box and whisker plots for Zn (variations in Zn concentration) for males and females were smaller than those for the other elements (Fig. 1). The formulae for both male and female groups, which were used to calculate the scores for discrimination, included the Zn concentrations: The Zn concentration appeared to be the largest contributor to the discriminant analysis because of the largest coefficients and very low odds ratios for Zn. In our previous report on several essential elements in scalp hair (Hotta et al., 2018), a significant decrease was found only in the Zn concentration of mild diabetics with a HbA1c value below 7%. To our knowledge, the decrease in Zn may be the most relevant among the essential elements for the onset of diabetes. A meta-analysis suggested that Zn may contribute to the management of hyperglycemia in chronic metabolic diseases as Zn supplementation decreases the glucose concentration and HbA1c value (Capdor et al., 2013). Furthermore, a recent meta-analytical study focusing on Zn, Cu, Cr and Mg levels clearly indicated a low level of Zn in subjects with type 2 diabetes

(Sanjeevi et al., 2018).

The Se concentrations in the scalp hair from the control males and females were similar to corresponding concentrations in the diabetic subjects, although the Se concentration in the male controls was slightly but significantly higher than that in the female controls (Table 1). In control subjects, the higher Se concentration in the males than in the females may be related to the higher concentration of Hg in males than in females (Table 1), as Hg and Se form nontoxic complexes in the plasma (Yoneda and Suzuki, 1997). The Hg concentration in the scalp hair of healthy subjects in Japan is higher in males than in females (Endo and Haraguchi, 2010; Kamakura, 1983), probably due to the higher level of fish consumption (Endo and Haraguchi, 2010; Endo et al., 2015). No differences were found in the Pb, Cd and As concentrations between the diabetic and control subjects (Table 1), although those concentrations were reported to be higher in the scalp hair of diabetics than controls (Kamakura, 1983).

Author contributions

Y.H. and T.E. contributed to the conception and design of the research. Y.F. and O.K. contributed to the collection of hair samples and analysis of the data. Y.H., K.H., and Y.F. contributed to the analysis and interpretation of the data. T.E. drafted the manuscript.

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Conflicts of interest

All authors (Hotta, Y., Fujino, R., Kimura, O., Fujii, Y., Haraguchi, K., and Endo, T.) do not have conflict interest including any financial, personal or other relationship with other people or organizations.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.obmed.2019.100106.

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