Regular Article

Essential and Non-essential Elements in Scalp Hair of Diabetics: Correlations with Glycated Hemoglobin (HbA1c)

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Essential elements (Ca, Mg, Zn, Cu, Fe, Cr, Mn and V) and non-essential elements (As, Cd, Hg and Pb) were measured in scalp hair samples of 45 diabetic subjects and 59 control subjects in Japan using inductively coupled plasma mass spectrometry. All diabetic subjects took insulin and/or antidiabetic agents, with glycated hemoglobin (HbA1c) ranging between 6.2 and 14.4%. The levels of Zn, Cu and Cr in the diabetic subjects (HbA1c>7) were significantly lower than those in the control subjects (p<0.05), and these concentrations decreased significantly with increases in HbA1c (p<0.01). The levels of Fe and Mg in the diabetic subjects were insignificantly lower, and the concentration of Fe decreased significantly with increases in HbA1c (p<0.05) and the concentration of Mn tended to decrease (p<0.10). In contrast, the concentration of As tended to increase with increases in HbA1c (p<0.10). The concentrations of other elements, such as Ca, Mn, V, Pb, Cd and Hg, in the diabetic subjects were similar to those of control subjects, and did not correlate with HbA1c. The average of estimated glomerular filtration rate (eGFR) in the diabetic subjects was 77.7±29.7mL/min/1.73m² with large variation (12.7–148mL/min/1.73m²), and previous study reported the increase of urinary excretion of Zn, Cr, Mn and Mg in diabetic subjects. The decreases of Zn, Cu, Cr, Fe and Mg concentrations in hair may reflect increased urinary excretion of these elements due to diabetic nephropathy.

Key words diabetes; scalp hair; glycated hemoglobin (HbA1c); zinc; chromium; copper

Diabetes mellitus is a serious and chronic metabolic disorder. Type 2 diabetes results from a disruption in glucose metabolism associated with a reduction in insulin production and utilization by tissues. Glycated hemoglobin (HbA1c) levels are used for screening and diagnostic purposes as HbA1c indicates the quality of blood glucose control over the previous 1–2 or 2–3 months. A HbA1c (%) value greater than 6.5 is a cut-off point for diabetic subjects and is highly prognostic for long-term diabetes-related complications such as nephropathy, retinopathy and neuropathy, and maintenance of the HbA1c value below 7.0 is a therapeutic guideline for the prevention of such complications of the Japan Diabetic Society. Estimated glomerular filtration rate (eGFR) is used for screening for nephropathy. The glomerular filtration rate (GFR) generally decreases as diabetes progresses, but the early stage of diabetic nephropathy is characterized by hyperfiltration (increase of GFR).

Deficiencies in trace elements such as Cr, Zn, Cu, Mg and Mn have been shown to predispose a person to glucose tolerance and to promote the development of diabetic complications. 2,3 Insulin resistance (IR) is defined as the condition in which insulin sensitive tissues become numb to insulin reception, and a Cr deficiency can induce IR. 2 Zn is known to synthesize insulin as well as stabilize insulin that has been stored in the beta cells of the pancreas. 5 Further, Zn has an important role in the secretion of insulin from the pancreatic beta cells. 5 Mg deficiency may be associated with IR and increase the risk for diabetes type 2. 5 A possible background to diabetic complications is the increase in the amount of reactive oxygen species (ROS) due to hyperglycemia. 6 Superoxide dismutase (SOD) nullifies the effects of superoxide by converting it to hydrogen peroxide, and Zn, Cu and Mn are cofactors for the isomers of the SOD enzyme. 7 Doddigalara et al. 8 analyzed Cr, Zn and Mg concentrations as well as SOD activity in the serum of control subjects and type-2 diabetic patients, and reported negative correlations between HbA1c and Cr concentration and between HbA1c and SOD activity in the diabetic patients.

Human scalp hair has been used to assess environmental and occupational exposure to toxic elements as well as to estimate and diagnose the nutritional and metabolic status of individuals. 9–13 As human scalp hair can be noninvasively sampled, and records the dietary history and physiological conditions over a much longer time-scale than does blood or urine: human scalp hair grows at a rate of approximately 1 cm per month, thus, scalp hair may record those conditions linearly along the length of the hair. 9,10 According to a review of hair analyses, 10 the levels of several essential elements, such as Ca, Mg, Cr, Zn and Mn, in the scalp hair of diabetic subjects were lower than those in the scalp hair of control subjects, while the levels of the toxic elements Pb, Cd and As were higher in the diabetic subjects than in the control subjects. However, some data for Mn and Mg previously reported in relation to diabetic subjects were controversial, 11 and nephropathy and hypertension, which are common complications of diabetes, affect the level of trace elements in the scalp hair. 8,14–16

There is no report on the correlation between HbA1c and the concentrations of essential and toxic metals in the scalp hair so far. In the present study, we investigate the relationships between HbA1c and the concentrations of 12 elements (Ca, Mg, Zn, Cu, Fe, Cr, Mn, V, As, Cd, Hg and Pb) in the scalp hair and between HbA1c and eGFR of diabetic patients in Japan.

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MATERIALS AND METHODS

Ethics Statement This research project and associated consent 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). Hair donors provided their written informed consent to participate in this study. The principles of Declaration of Helsinki were taken into consideration in each part of this study.

Sampling of Scalp Hair Scalp hair samples from 59 healthy donors living in Hokkaido, Aomori, Miyagi and Iwate Prefectures and the Tokyo Metropolitan area, Japan (control subjects), were collected during November 2009 and November 2015. As reported previously, control subjects are defined as donors of scalp hair who ate normal diets and declared to be healthy without any specific disease. The control subjects included 24 males (56.9±14.4 years, 37–83 years) and

| Patient | Gender | Age (years) | Height (cm) | Weight (kg) | BMI (kg/m²) | HbA1c (%) | eGFR (mL/min/1.73 m²) |
|---------|--------|-------------|-------------|-------------|-------------|-----------|----------------------|
| 1       | YHDM2  | M           | 39          | 176         | 74          | 24        | 8.3                  | 110.5                |
| 2       | RF14Y  | M           | 36          | 160         | 67          | 26.2      | 14.4                 | 148.1                |
| 3       | RF14A  | M           | 75          | 160         | 59          | 23        | 8.7                  | 51.1                 |
| 4       | RF14B  | M           | 54          | 147         | 43          | 19.9      | 8.2                  | 116.4                |
| 5       | RF14C  | F           | 69          | 153         | 84          | 35.9      | 8.3                  | 69.3                 |
| 6       | RF14G  | F           | 55          | 154         | 78          | 32.9      | 13.8                 | 108.7                |
| 7       | RF14H  | F           | 59          | 161         | 88          | 33.9      | 11.8                 | 99.3                 |
| 8       | RF14I  | M           | 43          | 176         | 106         | 34.2      | 8.6                  | 78.7                 |
| 9       | RF14J  | F           | 56          | 152         | 65          | 28.1      | 9.9                  | 110.9                |
| 10      | RF14N  | M           | 70          | 171         | 70          | 23.9      | 8.3                  | 43.7                 |
| 11      | RF14O  | M           | 55          | 163         | 73          | 27.5      | 8.2                  | 105.5                |
| 12      | RF14P  | F           | 84          | 149         | 65          | 29.3      | 8.8                  | 37.7                 |
| 13      | RF14Q  | M           | 77          | 163         | 58          | 21.8      | 11.3                 | 12.7                 |
| 14      | RF14S  | M           | 61          | 174         | 70          | 23.1      | 12.9                 | 116.7                |
| 15      | RF14T  | F           | 83          | 159         | 85          | 33.6      | 8.1                  | 45.3                 |
| 16      | RF14U  | F           | 49          | 158         | 52          | 20.8      | 10.1                 | 107.2                |
| 17      | RF14V  | M           | 40          | 168         | 71          | 25.2      | 9.6                  | 129.4                |
| 18      | RF14W  | M           | 65          | 157         | 57          | 23.1      | 8                    | 80.2                 |
| 19      | RF14X  | M           | 67          | 163         | 59          | 22.2      | 8.7                  | 49.8                 |
| 20      | RF14Y  | M           | 66          | 160         | 65          | 25.4      | 10.2                 | 98.3                 |
| 21      | RF14Z  | M           | 47          | 168         | 75          | 26.6      | 12                   | 69.6                 |
| 22      | RF14AA | F           | 66          | 152         | 60          | 25.9      | 9.8                  | 140.2                |
| 23      | RF14CB | F           | 76          | 155         | 83          | 34.5      | 9.4                  | 58.4                 |
| 24      | RF14GG | M           | 54          | 177         | 91          | 29.3      | 7.8                  | 44.7                 |
| 25      | H31    | M           | 50          | 168         | 72          | 17.7      | 7.5                  | N                    |
| 26      | DIM1   | M           | 69          | 165         | 67          | 24.6      | 6.6                  | 56.3                 |
| 27      | DIM3   | F           | 66          | 150         | 58          | 25.8      | 6.7                  | 84.5                 |
| 28      | DIM4   | M           | 67          | 147         | 62          | 28.7      | 6.9                  | N                    |
| 29      | RF14D  | F           | 79          | 148         | 53          | 24.2      | 6.7                  | 85.5                 |
| 30      | HDM2   | F           | 65          | 147         | 48          | 22.2      | 6.8                  | N                    |
| 31      | YHDM3  | M           | 86          | 164         | 63          | 23.4      | 6.6                  | 44.3                 |
| 32      | YHDM4  | F           | 62          | 158         | 62          | 24.8      | 6.5                  | 78.1                 |
| 33      | H34    | F           | 66          | 145         | 50          | 23.8      | 6.4                  | N                    |
| 34      | YHDM1  | M           | 75          | 156         | 57          | 23.4      | 6.2                  | 23.0                 |
| 35      | H30    | M           | 56          | 170         | 74          | 19.4      | 6.2                  | N                    |
| 36      | YHDM6  | M           | 67          | 165         | 63          | 23.1      | 6.2                  | 67.6                 |
| 37      | HDM1   | M           | 77          | 162         | 68          | 25.9      | 6.2                  | N                    |
| 38      | H37    | F           | 80          | 158         | 45          | 18        | 8.5                  | 59.3                 |
| 39      | RF14HH | M           | 52          | 167         | 66          | 23.7      | 12.7                 | 95.2                 |
| 40      | RF14I  | F           | 73          | 148         | 37          | 16.8      | 14.3                 | 82.1                 |
| 41      | RF14J  | F           | 75          | 159         | 50          | 20        | 7.9                  | 92.7                 |
| 42      | RF14K  | M           | 75          | 159         | 54          | 21.5      | 14.3                 | 103.9                |
| 43      | RF14L  | M           | 85          | 158         | 44          | 17.6      | 8.2                  | 71.1                 |
| 44      | RF14M  | M           | 84          | 159         | 57          | 22.6      | 7.5                  | 71.4                 |
| 45      | RF14N  | M           | 71          | 173         | 73          | 24.3      | 11                   | 84.3                 |

| Mean    | 66.3   | 159.6 | 64.7 | 24.9 | 8.89 | 77.7 |
| S.D.    | 11.9   | 8.4   | 14.0 | 4.8  | 2.32 | 29.7 |

N: not available.
35 females (64.2 ± 16.3 years, 33–83 years), with an average age of 61.2 ± 15.8 years. The HbA1c values of 13 male and 20 female subjects were 5.1 ± 0.3 (4.7–6.1) and 5.1 ± 0.3 (4.3–5.9), respectively, but others’ were unknown.

Scalp hair samples from 45 diabetics living in Hokkaido Prefecture and the Tokyo Metropolitan, Japan, were collected during November 2009 and April 2016. We collected hair sample from the scalp of patients where possible, and did not collect the samples from a particular region and from a specific length from the scalp. Patient who showed a sudden decrease in body weight within at least one month before collection of hair sample. Information on the diabetic subjects collected is listed in Table 1. The diabetic subjects included 29 males (63.1 ± 14.0 years) and 16 females (67.9 ± 10.4 years) with an average age of 66.3 ± 11.9 years (n = 45). The body mass index (BMI) of total, male and female subjects was 24.9 ± 4.8, 24.3 ± 3.9, and 26.0 ± 5.9 kg/m², respectively, and the HbA1c was 8.9 ± 2.3, 8.9 ± 2.4 and 8.9 ± 2.5%, respectively. The HbA1c of 3 subjects exceeded 14%. The average of estimated glomerular filtration rate (eGFR) was 77.7 ± 29.7 mL/min/1.73 m². No gender-related differences were observed among the characteristics. Among the 45 diabetic subjects, two were diagnosed with type 1 diabetes (No. 38 and 41 in Table 1). All hair samples were packed in paper or polyethylene bags and stored at room temperature until analysis.

Analyses of Elements in Scalp Hair The hair samples were washed with a mixture of methanol and chloroform and the weighted hair (0.020–0.025 g) was digested by 0.5 mL nitric acid, ultrapure grade (Kanto Kagaku Co., Inc., Tokyo, Japan) at 100°C for 1 h. After cooling to room temperature and adjusting its gravimetric volume, the resultant product was used for mineral analysis. Twelve elements were measured by inductively coupled plasma mass spectrometry (Agilent-7700 ICP-MS, Agilent Technology, Santa Clare, CA, U.S.A.). The element concentrations were expressed as mg/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 Ca, Mg, Zn, Cu, Fe, Cr, Mn, V, As, Cd, Hg and Pb were in good agreement with the certified values. Recoveries of the elements from NIES No. 13 and NCS No. ZC81002b were 76.7 to 105% and 76.3 to 114%, respectively (Supplementary Tables S1 and S2).

Statistical Analyses Data were expressed as the mean ± standard deviation (S.D.). Data were analyzed by Tukey-Kramer test or Scheffe’s F-test using the Statcel 2 program (add-in soft ware on Excel, OMS, Japan), with a value of...
Table 2. In the case of control subjects whose HbA1c value was observed in each element between male and female control subjects and between male and female patients. 7% were significantly lower than those in the control subjects (n=12) or above 7% (n=33) as well as those from the control subjects (n=59). The levels of Zn (n=33), Cu (n=30) and Cr (n=32) in the diabetic subjects with a HbA1c above 7% were significantly lower than those in the control subjects (n=59, p<0.05). As data not shown, no significant difference was observed in each element between male and female control subjects and between male and female patients.

Figures 1A and 1B show the relationships between HbA1c and each element of control and diabetic subjects shown in Table 2. In the case of control subjects whose HbA1c value were unknown, these values of male and female subjects were estimated to be 5.2 and 5.3, respectively. The concentrations of Zn, Cu, Cr and Fe decreased linearly or exponentially with increases in HbA1c (p<0.01 or p<0.05), and the concentration of Mg tended to decrease (p<0.10). In contrast, the concentration of As tended to increase with increases in HbA1c (p<0.10). The concentrations of other elements, such as Ca, Mn, V, Pb, Cd and Hg, did not correlate with HbA1c. As data not shown in figure, the ratios of Ca to Mg and Zn to Cu were 13.4±6.5 and 10.5±5.6, respectively, and did not correlate with HbA1c (R²=0.026 and 0.0224, respectively).

Table 3 shows a summary of eGFR from 39 diabetic subjects. The eGFR values of 24 patients were below 90 mL/min/1.73 m² (cut off point), with large variation (12.7–148 mL/min/1.73 m²). The three highest eGFRs values (148, 140, and 129 mL/min/1.73 m²) were found in the diabetic subjects with high HbA1c values being 14.4, 9.8 and 9.6, respectively. Positive correlation was found between eGFR and HbA1c (R²=0.161, p<0.01), mainly reflecting the data of diabetic subjects with higher eGFR and HbA1c, and no correlation was found between eGFR and Zn, Cu, Cr, Fe or Mg concentration.

DISCUSSION

Previous studies (reviewed by Mooradian et al.) suggested that the onset of diabetes is related to deficiencies in essential trace elements such as Zn, Cu, Mn and Mg. To our knowledge, we are the first to show negative correlations between HbA1c and the Zn, Cr and Cu concentrations (p<0.01), and a negative tendency between HbA1c and the Mg concentration (p<0.10) (Fig. 1A), in hair samples from diabetic subjects. In addition, a negative correlation was also found between HbA1c and the Fe concentration (p<0.05). The Zn and Cr concentrations in the hair samples from diabetic subjects were significantly lower than observed in the control subjects (Table 2) and decreased with increases in HbA1c (Fig. 1A). The Mg concentration also tended to decrease with increases in HbA1c (p<0.10, Fig. 1A), although the average Mg concentrations in the diabetic and control subject were similar (Table 2). Kazi et al. reported that the levels of Zn, Cr and Mg in the hair and blood were significantly lower in diabetic subjects than in control subjects, while the urinary concentrations of those elements were significantly higher in the diabetic subjects. Chen et al. reported that the Zn and Mg concentrations in plasma and erythrocytes were lower in diabetic subjects than in control subjects, while the urinary excretion of Zn and Mg was higher in the diabetes subjects. Mita et al. also reported the higher urinary excretion of Cr in diabetic mice. Considered together, the lower concentrations of Zn, Cr and Mg observed in the hair samples from diabetic subjects (Fig. 1A) could reflect higher levels of urinary excretion of Zn, Cr and Mg. A lower Ca concentration in the hair and a higher urinary excretion of Ca were reported in diabetic subjects, but we did not find any tendency for the Ca concentration in the hair to decrease with increases in HbA1c (Fig. 1A).

The Cu concentration in the hair samples from diabetic subjects after the rejections of three outliers (n=30) was significantly lower than that in the control subjects (Table 2) and decreased with increases in HbA1c (Fig. 1A). However, the results of previous studies on Cu were controversial. Tanega et al. reported a lower Cu concentration in the hair from diabetic subjects than in that from control subjects and a similar level of urinary excretion of Cu in the diabetic and control subjects. Kazi et al. reported that the Cu concentrations in the scalp hair and urine of diabetic subjects were slightly higher than those of control subjects. Mooradian et al. pointed out the controversial data regarding the tissue distribution.
of Cu in diabetic subjects.

The level of Fe in the scalp hair samples from the diabetic subjects decreased with increases in HbA1c (p<0.05; Fig. 1A), whereas the level of Mn in the scalp hair samples from the diabetic subjects was similar to that in the control subjects (Table 2), and did not decrease with increases in HbA1c (Fig. 1B). Contrary to our results, Kazi et al. reported higher levels of Fe in the scalp hair of diabetic subjects than in control subjects with lower levels of Fe excretion in the urine of diabetic subjects, and the lower levels of Mn in the hair of diabetic subjects than in control subjects. The reasons for this discrepancy remain open at the present.

Taneja et al. reported a significant negative correlation between the BMI of diabetic patients and the Zn concentration in their hair and a significant positive correlation between the BMI and Zn concentration in their urine. They also reported a positive correlation between the BMI and the Cu concentration in their hair as well as in their urine. In our data, the Zn concentration in the hair samples from diabetic subjects tended to decrease with increases in their BMI (p<0.10), but no correlation was found between the BMI and concentration of Cu or other elements (p>0.10).

V and V-containing compounds have been assessed for use in the treatment of diabetic patients. However, the V concentration in the hair samples from the diabetic subjects was similar to that in the control subjects (Table 2). Therefore, diabetes may not be related to a deficiency in V.

The concentrations of the toxic elements As, Cd, Hg and Pb in the scalp hair samples from the diabetic subjects were similar to those in the control subjects (Table 2), but the As concentration tended to increase with increases in HbA1c (p<0.10, Fig. 1B). Afridi et al. reported that the As, Cd and Pb concentrations in the scalp hair, blood and urine of diabetic subjects were higher than those of control subjects, and these toxic elements may have a role in the development of diabetes mellitus (a diabetogenic effect).

The average eGFR was 77.7 mL/min/1.73 m² with large variation (SD was 29.7), and no correlation was found between eGFR and Zn, Cu, Cr, Fe or Mg concentration. The reason for no correlation is that the present study included the diabetic subjects with extremely low values of eGFR and high values of eGFR. Unfortunately, no information is available on the relation between the eGFR value and urinary excretion of these elements due to diabetic nephropathy. On the other hand, the concentrations of other elements, such as Ca, Mn, V, Pb, Cd and Hg, in the diabetic subjects were similar to those of control subjects.

CONCLUSION

We measured 12 elements in the scalp hair from diabetic and control subjects, and investigated the relationships between the elements and HbA1c. The Zn, Cr, Cu and Fe concentrations decreased with increases in HbA1c (p<0.05 or 0.01) and the Mg concentration tended to decrease (p<0.10), while the As concentration tended to increase with increases in HbA1c (p<0.10). Although the average of eGFR value in diabetic subjects was below 90 mL/min/1.73 m² (cut-off point), but the eGFR values of some subjects exceeded 120 mL/min/1.73 m². The decreases in the concentrations of these elements in the hair may reflect the increases in the urinary excretion of these elements due to diabetic nephropathy.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

REFERENCES

1) Hiragushi K, Sugimoto H, Shikata K, Yamashita T, Miyatake N, Shikata Y, Wada J, Kumagai I, Fukushima M, Makino H. Nitric oxide system is involved in glomerular hyperfiltration in Japanese normo- and micro-albuminuric patients with type 2 diabetes. *Diabetes Res. Clin. Pract.*, 53, 149–159 (2001).
2) Mooradian AD, Failla M, Hoogwerf B, Maryniuk M, Wyle-Rosett J. Selected vitamins and minerals in diabetes. *Diabetes Care*, 17, 464–479 (1994).
3) Chen MD, Lin PY, Tsou CT, Wang JJ, Lin WH. Selected metals status in patients with noninsulin-dependent diabetes mellitus. *Biol. Trace Elem. Res.*, 50, 119–124 (1995).
4) Kinlaw WB, Levine AS, Morley JE, Silvis SE, McClain CJ. Abnormal zinc metabolism in type II diabetes mellitus. *Am. J. Med.*, 75, 273–277 (1983).
5) Huerta MG, Roemmich JN, Kington ML, Bovbjerg VE, Weltman AL, Holmes VF, Patrie JT, Rogol AD, Nadler JL. Magnesium deficiency is associated with insulin resistance in obese children. *Diabetes Care*, 28, 1175–1181 (2005).
6) Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ. Res.*, 107, 1058–1070 (2010).
7) Machata E, Adamcz T, Insue M, Yano M, Shimomura H, Shiba T, Yamakado M, Insue T, Suzuki S, Kawaguchi T, Okabe E. High blood superoxide dismutase (SOD) states in patients with diabetes mellitus—Dependence on extracellular (EC)-SOD. *J. Jpn. Diab. Soc.*, 44, 935–941 (2001).
8) Dodiagara Z, Parvez I, Ahmad J. Correlation of serum chromium, zinc, magnesium and SOD levels with HbA1c in type 2 diabetes.
A cross sectional analysis. Diabetes Metab. Syndr., 10 (Suppl. 1), S126–S129 (2016).

9) Petzke KJ, Fuller BT, Metges CC. Advance in natural stable isotope ratio analysis of human hair to determine nutritional and metabolic status. Curr. Opin. Clin. Metab. Care, 13, 532–540 (2010).

10) Wołowiec P, Michalak I, Chojnacka K. Relationships among mercury concentration, and stable isotope ratios of carbon and nitrogen of amino acids in scalp hair from whale meat eaters and heavy fish eaters. J. Inorg. Biochem., 104, 303–308 (2008).

11) Afridi HI, Kazi TG, Kazi N, Jamali MK, Arain MB, Jalbani N. Metal toxic levels in scalp hair of infants and children. Biol. Trace Elem. Res., 62, 255–264 (1998).

12) Chojnacka K, Michalak I, Zielinska A, Gorecki H, Gorecki H. Inter-relation between elements in human hair: The effect of gender. Ecotoxicol. Environ. Saf., 73, 2022–2028 (2010).

13) Endo T, Hayasaka M, Ogasawara H, Hotta Y. High toxic metal levels in scalp hair of infants and children. J. Pediatr. Child Health, 41, 823–838 (1983).

14) Kamakura M. A study of the characteristics of trace elements in the hair of Japanese. Reference values and element patterns for determining normal levels. Nippon Eiseigaku Zasshi, 38, 823–838 (1983).

15) Ochi A, Ishimura E, Tsujimoto Y, Kakiya R, Tabata T, Mori K, Shoji T, Yasuda H, Nishizawa Y, Inaba M. Trace elements in the hair of hemodialysis patients. Biol. Trace Elem. Res., 143, 825–834 (2011).

16) Ono M, Nishigaki Y. Fasting plasma glucose and glycosylated hemoglobin levels in mass screening. JARM, 49, 137–141 (2000).

17) Endo T, Hayasaka M, Ogasawara H, Kura T, Tarumi T, Muramatsu H, Endo T. Nutritional assessment using stable isotope ratios of nitrogen and carbon in scalp hair of patients who received enteral nutrients. Jpn. J. Pharm. Health Care Sci., 42, 151–159 (2016).

18) Petzke KJ, Fuller BT, Metges CC. Advance in natural stable isotope ratio analysis of human hair to determine nutritional and metabolic status. Curr. Opin. Clin. Metab. Care, 13, 532–540 (2010).

19) Afridi HI, Kazi TG, Kazi N, Jamali MK, Arain MB, Jalbani N, Kandhro GA. Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients. Biol. Trace Elem. Res., 122, 1–18 (2008).

20) Mita Y, Ishihara K, Ishiguro M, Takeda M, Hattoni R, Murakami K, Yamada A, Yasumoto K. Elevated urinary Cr loss induces a reduction in renal Cr concentration and the negative Cr balance in streptozotocin-induced diabetic mice. J. Nutr. Sci. Vitaminol., 54, 303–308 (2008).

21) Taneja SK, Mahajan M, Gupta S, Singh KP. Assessment of copper and zinc status in hair and urine of young women descendants of NIDDM parents. Biol. Trace Elem. Res., 62, 255–264 (1998).

22) Thompson KH, Orvig C. Vanadium in diabetes: 100 years from phase 0 to phase I. J. Inorg. Biochem., 100, 1925–1935 (2006).

23) Afridi HI, Kazi TG, Kazi N, Jamali MK, Arain MB, Jalbani N, Baig JA, Sarraz RA. Evaluation of status of toxic metals in biological samples of diabetes mellitus patients. Diabetes Res. Clin. Pract., 80, 280–288 (2008).

24) Viktorinova A, Tosevova E, Krizko M, Durackova Z. Altered metabolism of copper, zinc, and magnesium is associated with increased levels of glycerated hemoglobin in patients with diabetes mellitus. Metabolism, 58, 1432 (2009).

25) Kamakura M. A study of the characteristics of trace elements in the hair of Japanese. Reference values and element patterns for determining normal levels. Nippon Eiseigaku Zasshi, 38, 823–838 (1983).

26) Ochi A, Ishimura E, Tsujimoto Y, Kakiya R, Tabata T, Mori K, Shoji T, Yasuda H, Nishizawa Y, Inaba M. Trace elements in the hair of hemodialysis patients. Biol. Trace Elem. Res., 143, 825–834 (2011).

27) Chojnacka K, Michalak I, Zielinska A, Gorecki H, Gorecki H. Inter-relation between elements in human hair: The effect of gender. Ecotoxicol. Environ. Saf., 73, 2022–2028 (2010).

28) Skalnaya MG, Tinkov AA, Demidov VA, Serhryansky EP, Nikonorov AA, Skalny AV. Age-related differences in hair trace elements: a cross-sectional study in Orenburg, Russia. Ann. Hum. Biol., 18, 1–7 (2015).

29) Dlugaszek M, Szopa M, Rzeszotarski J, Karbowiak P. Magnesium, calcium and trace elements distribution in serum, erythrocytes, and hair of patients with chronic renal failure. Magnes. Res., 21, 109–117 (2008).