2024年3月11日発行

Determination of Zinc Sufficiency via the Direct Assessment of Fingertip Blood Samples

Yuka MIYAJIMA[1], Makoto NODERA[2], and Shuichi ADACHI[3]

[1] *Department of Clinical Laboratory Science, Nihon Institute of Medical Sciences, Moroyama, Saitama, Japan, e-mail: y-miyajima@nims.ac.jp*

[2] *Department of Clinical Laboratory Science, Nihon Institute of Medical Sciences, Moroyama, Saitama, Japan, e-mail: m-nodera@nims.ac.jp*

[3] *Department of Food and Nutrition Science, Sagami Women's University, Sagamihara, Japan, e-mail: s-adachi@star.sagami-wu.ac.jp (mmfnnk67@gmail.com)*

相模女子大学紀要　　VOL.87（2023年度）

Determination of Zinc Sufficiency via the Direct Assessment of Fingertip Blood Samples

Yuka MIYAJIMA[1], Makoto NODERA[2], and Shuichi ADACHI[3]

[1] *Department of Clinical Laboratory Science, Nihon Institute of Medical Sciences, Moroyama, Saitama, Japan, e-mail: y-miyajima@nims.ac.jp*

[2] *Department of Clinical Laboratory Science, Nihon Institute of Medical Sciences, Moroyama, Saitama, Japan, e-mail: m-nodera@nims.ac.jp*

[3] *Department of Food and Nutrition Science, Sagami Women's University, Sagamihara, Japan, e-mail: s-adachi@star.sagami-wu.ac.jp (mmfnnk67gmail.com)*

Abstract

Zinc (Zn) sufficiency status is commonly determined clinically by serum or plasma zinc concentrations from venous blood collection. To investigate whether the measurement of whole blood Zn concentration using simple and less invasive fingertip blood samples reflects the Zn sufficiency in the body, we first measured the tissue and whole blood Zn concentrations during Zn deficiency and recovery in rats, followed by the same measurements in the fingertip blood samples of young females. Ten microliter of blood sample was diluted, hemolyzed in ultrapure water, and analyzed (50 samples/2 h) without acid digestion or any other pretreatment. Zn restriction in young rats rapidly resulted in Zn deficiency symptoms and a marked decrease in serum Zn concentrations; however, there were no significant changes in Zn concentrations in the liver, muscle tissue, or whole blood from the rat tail vein. In young Japanese women with no health problems (N = 69), average fingertip blood Zn concentration was 793.5 μg/dL (95% confidence interval [C.I.]: 717.0–870.1 μg/dL) and average serum Zn concentration was 76.4 μg/dL (95% C.I.: 78.7–74.2 μg/dL), although no correlation was observed between the two. Average estimated Zn concentration in blood cells was 1271.6 μg/dL (95% C.I.: 1144.0–1399.1 μg/dL), suggesting that individual differences were predominantly influenced by the Zn concentration in blood cells. Whole-blood Zn concentration assay using small amounts of fingertip blood is feasible as a simple evaluation method. Further study will be conducted in all age groups, including males, and the association with variables will be analyzed.

Keywords：zinc, fingertip, flameless atomic absorption spectrometry, screening

Introduction

This study proposes a screening method for the identification of zinc (Zn)- deficient individuals. Recent research has made significant progress in elucidating the physiological functions and clinical etiology of Zn. This study validates a simple and widely available Zn assay for public health application.

The physiological importance of Zn as an essential trace element has been recognized, and its association with various diseases has been demonstrated.[1, 2, 3] Various methods have been proposed and validated to determine the sufficiency of Zn in the body.[4, 5, 6, 7, 8, 9, 10, 11, 12] Measurement of serum or plasma Zn concentration is widely as a clinical test, and recently, a test method based on the expression levels of Zn transporters as indicators has also been proposed.[13, 14, 15] However, because the measurement of plasma or serum Zn concentration requires venous blood sampling, it should only be applied to clinical cases deemed necessary by a physician and should not be performed for the general population. There are few opportunities for Zn testing in the general population, except for research purposes. In addition, the sensitivity and specificity of this indicator have been pointed out to be low for diagnosis based on plasma or serum Zn concentration and for the study of Zn sufficiency in the body.[14, 16, 17, 18, 19] Zn deficiency is a common problem in both developing and developed countries. Moreover, Zn deficiency is a nutritional challenge in both developing and developed countries, with higher rates reported in young women and older men in developed countries, as determined by the plasma or serum Zn levels.[12, 13, 20, 21, 22] Therefore, there is public health value in identifying Zn-deficient individuals in the population, implementing nutritional education and other interventions, and evaluating their public health value. For this purpose, a simpler and more accurate Zn assay than venous blood sampling is required. The purpose of this study was to test the utility of measuring the Zn concentration in whole blood in trace amounts, such as in the fingertip blood. Here, we experimentally created Zn deficiency in rats, examined the relationships among the tail vein blood, serum, and organ Zn concentrations in rats, and also determined the relationship between fingertip blood and serum Zn concentrations in young Japanese women.

PART 1: Changes in Blood and Tissue Zn Concentrations During the Onset of Zn Deficiency and Recovery in Rats

To observe the relationship between blood and tissue Zn concentrations during the onset of Zn deficiency and recovery, deficient rats were created via the ingestion of a Zn-deficient diet, followed by return to a normal diet, and blood, heart, liver, and muscle Zn concentrations were measured until the recovery phase. Blood Zn concentrations were analyzed in the tail vein spot blood and serum at autopsy.

Experimental Design

Experimental Animals and Conditions

Five-week-old female rats (F344/Nslc, N = 66, weight 107.6 ± 5.0 g) were purchased from Japan SLC, Inc, Hamamatsu, Shizuoka, Japan. Both the feed and water (ultrapure water) were provided ad libitum. The rats were reared individually, and feces and urine were separated from the rearing environment using a bed net in the cage. The animal room was maintained at a temperature of 23–25℃ and humidity of 45–64%, and the light/dark cycle was adjusted with a 12-h timer.

Experimental Groups

Three groups were established: Zn-deficient (D), Zn-deficient recovery (R), and control (C) groups. The standard diet was AIN-93G (D10012G, Research Diet Inc, New Brunswick, USA) and Zn-deficient diet was AIN-93G without Zn (D19488, Research Diet Inc,).[23] After one week of acclimation and rearing, the animals were divided into groups C, D, and R, with 30, 24, and 12 animals, respectively. The rats in group C were maintained on a standard diet for 12 weeks. Body

weight and whole-blood Zn levels were measured weekly. Six animals in each group were dissected every four weeks for serum Zn measurement and observation of tissue changes.

Examinations

In this experiment, Zn levels in whole blood and serum were measured in rats of groups C, D, and R. Tissue Zn concentrations were measured in the liver, heart, and thigh muscles. Histopathological examination of the duodenum was also performed to observe the histopathological changes caused by Zn deficiency and re-administration.

Zn Concentration in Whole Blood

In groups C, D, and R, the rats were examined every week from the beginning of the experiment to 12 weeks. Ten microliters of blood was collected from the tail veins of rats under light anesthesia. Blood samples were immediately diluted 100-fold with ultrapure water. The amount of Zn in whole blood was measured within 6 h of blood collection using an atomic absorption spectrophotometer (AA240; Agilent, Australia). A flameless atomic absorption spectrophotometer using the furnace method was used. The time and temperature settings for the flameless atomizer are listed in Table 1.

Table 1: Settings for the graphite furnace atomizer equipped with atomic absorption spectrophotometer to determine zinc concentration in diluted whole blood.

step	temperature(C)	time(sec)	argon gas flow(L/min)
1	85	10	0.3
2	95	30	0.3
3	100	10	0.3
4	120	5	0.3
5	300	4	0.3
6	350	8	0.3
7	400	5	0.3
8	500	2	0.3
9	2000	0.8	0.3
10	2000	2	0.3
11	2200	2	0.3

Zn Concentration in Serum

Groups C and D were evaluated after collecting blood samples from six rats in each group 4, 8, 10, and 12 weeks after the start of the experiment. Blood samples were collected from the posterior vena cava of anesthetized rats after the intraperitoneal administration of pentobarbital. For group R, blood samples were collected in the same manner 2 and 4 weeks after the re-administration of the standard diet. The collected whole blood was allowed to stand for 30 min and centrifuged to obtain the serum. The amount of Zn in the serum was measured in the same manner as that in the whole blood.

Zn Concentration in Tissues

Liver, heart, and thigh muscles were removed after the blood was drawn from the posterior vena cava. The samples were stored at −70 ℃ until further analysis. Approximately 100 mg tissue was cut out from the removed liver, heart, and femoral muscles. The excised tissue and nitric acid were placed in a teflon vessel. The vessels were heated on a graphite hot plate and subjected to wet degradation. These pretreatments were performed using the Actac's acid circulation decomposition system Ecopre system (OD-SYS24). The amount of Zn in the tissue was measured in the same manner as that in the whole blood. [24, 25, 26, 27, 28]

Histological Changes

After collecting blood from the posterior vena cava, the duodenum was removed, fixed in 10% neutralized formalin, paraffin-embedded, and pathological specimens were prepared using standard methods. Histopathological changes in the duodenum were then observed via hematoxylin and eosin staining.

Statistical Processing

All data were analyzed using the statistical software SPSS Statistics Ver. 25 and are presented as the mean ± standard deviation (SD). $p < 0.05$ was considered to be significantly different. Student's t-test and one-way analysis of

variance were used to compare the significant differences among groups.

Ethics

This study was approved by the Research Ethics Committee of the Sagami Women's University Experimental Animal Committee (April 20, 2017; approval no. 2017-02).

Results

Change in Body Weight

Body weights of mice in groups C, D, and R during the experimental period are shown in Fig. 1. Groups D and R, which received Zn-deficient diet, did not gain weight until the 8th week, while group R, which switched to the standard diet, gained weight significantly, but did not reach that of group C even on the 12th week.

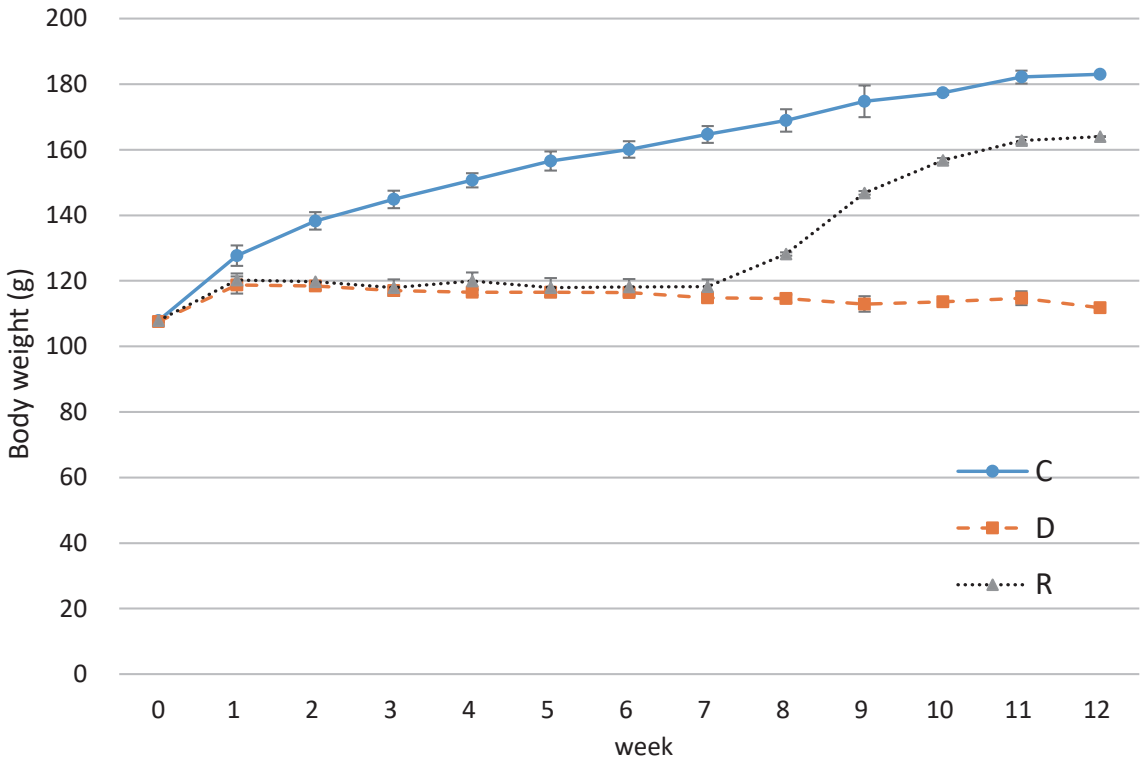

Figure 1: Body weight (mean and S.D. grams) change of female F344 rats fed zinc deficient diet (D: deficient diet group, R: recovery group, C: control)

Macroscopic Findings

No abnormal findings were observed in group C during the experimental period. Group D showed inflammation at the corners of the mouth, nose, and periorbital area in 3 of the 36 (8.3%) cases in the third week of the experiment. One of the 36 (2.8%) cases showed alopecia on the back in the fourth week. On week 8, more than half of the subjects in D group showed symptoms, such as dermatitis and alopecia, which are characteristic of Zn deficiency. In group R, inflammation at the corners of the mouth and around the eyelids, alopecia, and dermatitis at the ends of the extremities improved from that on the second week after reintroduction of the standard diet. In group R, inflammation at the corners of the mouth and around the eyelids, alopecia, and dermatitis at the ends of the limbs improved from that on the second week after reintroduction of the standard diet.

Microscopic Findings

In group D, 8 weeks after the start of the experiment, the border of the crypts was indistinct in B compared to group C: A. Immature cells increased in the crypts, and thickening of the crypts was observed. Group R showed a clear increase in the number of immature cells in the crypts and thickening of the crypts compared to group D, which continued to receive a Zn-deficient diet.

Zn Concentration in Whole Blood

Zn concentration in the tail vein blood was 370.4 ± 4.9 μg/dL (average ± SD) in group C. Zn concentration in group D showed a significant decrease to 326.2 μg/dL in the first week and continued to decrease to 301.1 μg/dL in the sixth week, after which it increased slightly but remained lower than group C (347.1 ± 8.6 μg/dL). Group R had the same level as group C at 8 weeks, and higher levels at 10 and 12 weeks (379.0 ± 15.3 μg/dL), although the difference was not significant (Fig. 2).

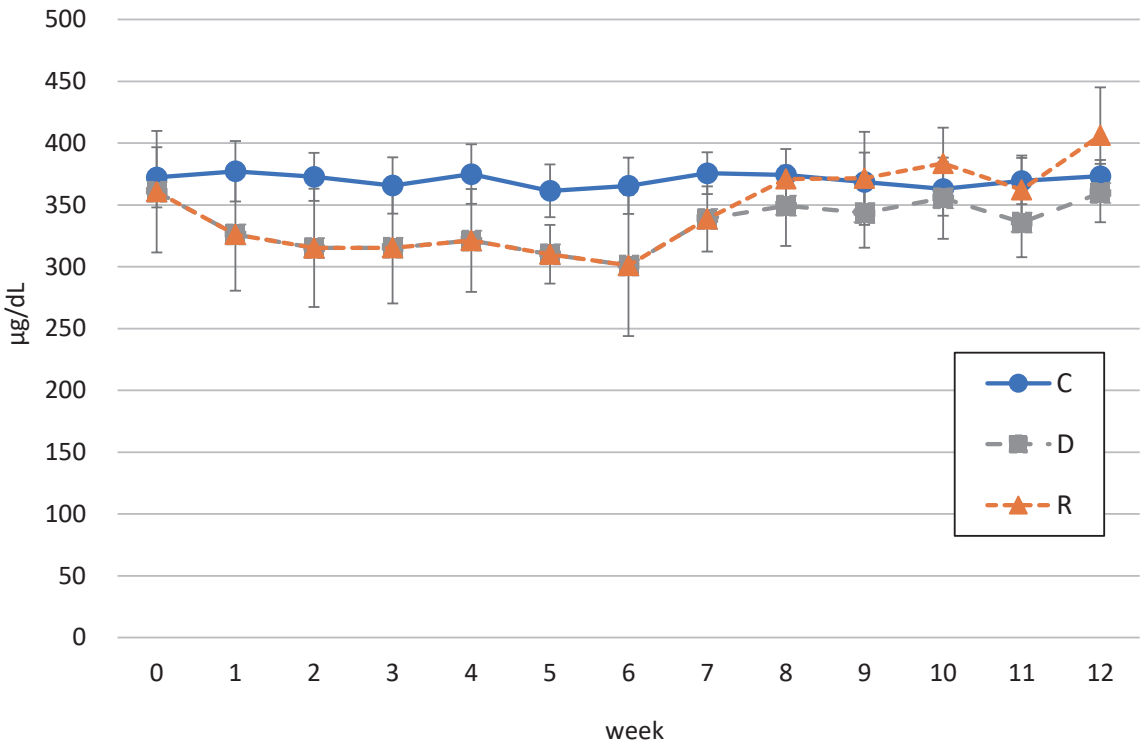

Figure 2: Zinc concentration of micro suction blood (mean and S.D. μg/dL) in rats fed zinc deficient diet (D: deficient diet group, R: recovery group, C: control)

Zn Concentration in Serum

Serum Zn concentrations in groups C, D, and R are shown in Fig. 3. The mean serum Zn concentrations in group C ranged from 100.9 μg/dL at the beginning of the experiment to 94.8, 94.3, 102.2, and 114.2 μg/dL. Serum Zn concentrations in group D remained significantly lower than those in group C at 21.1, 25.1, 26.7, and 24.2 μg/dL after four weeks of the experiment. Serum Zn concentration in group R was not significantly different from that of group C at 10 weeks, two weeks after returning to the standard diet.

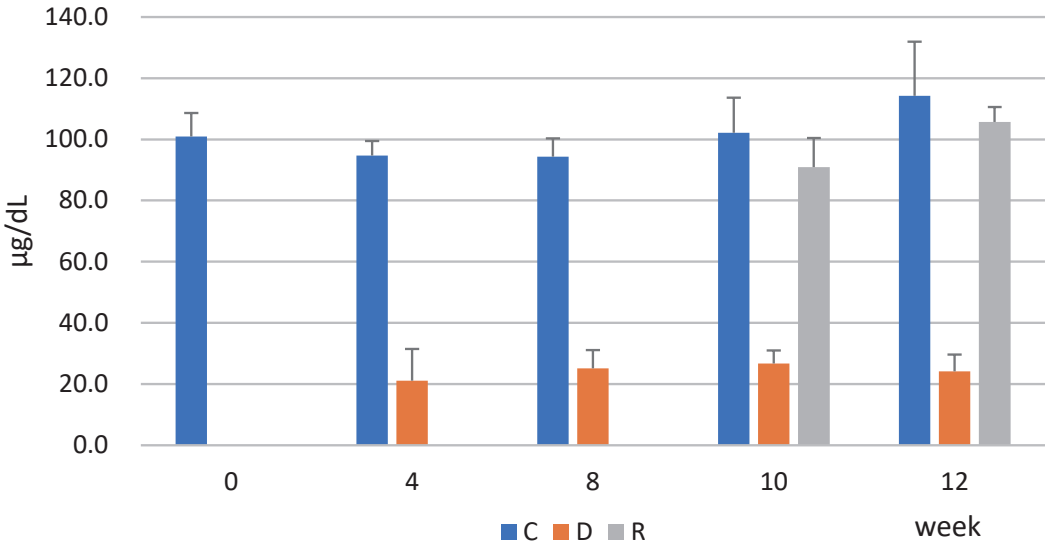

Figure 3: Serum zinc concentration (mean and S.D. μg/dL) of rats fed zinc deficient diet (D: deficient diet group, R: recovery group, C: control)

Zn Concentration in Blood Cells

Based on the tail vein and serum Zn concentrations, blood cell Zn concentration was calculated based on the hematocrit values. As shown in Fig. 4, throughout the 4–12-week experimental period, blood Zn concentrations in the control, Zn-deficient, and recovery groups ranged from 609–691 μg/dL, with no statistically significant differences between the groups. Blood Zn concentrations in the deficient and recovery groups tended to be higher than those in the control group.

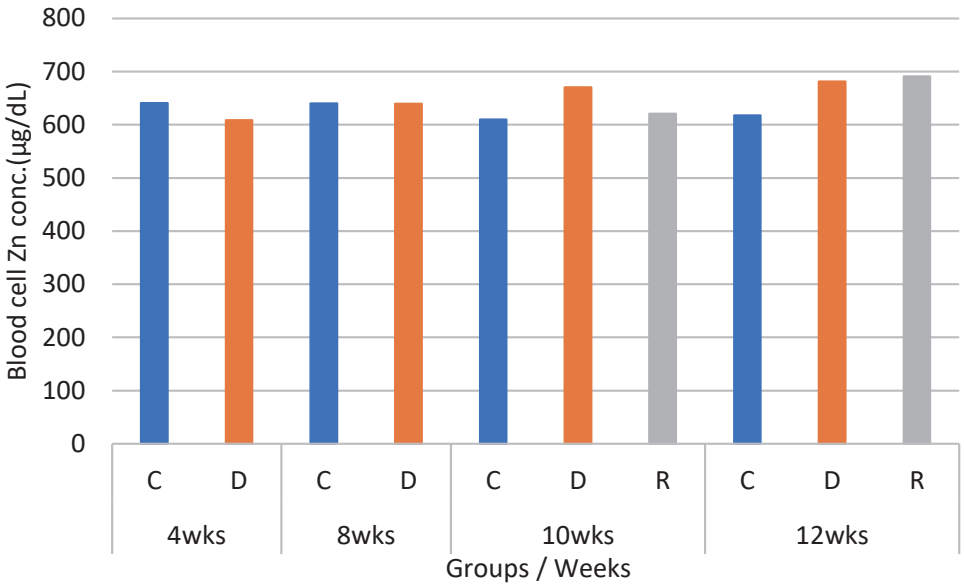

Figure 4: Estimated zinc concentration (mean μg/dL) in blood cell fraction of rats fed zinc deficient diet (D: deficient diet group, R: recovery group, C: control)

Zn Concentration in Tissues

Zn concentrations in the liver, heart, and thigh muscles are shown in Fig. 5,6 and 7, expressed as micrograms of Zn per gram of fresh tissue.

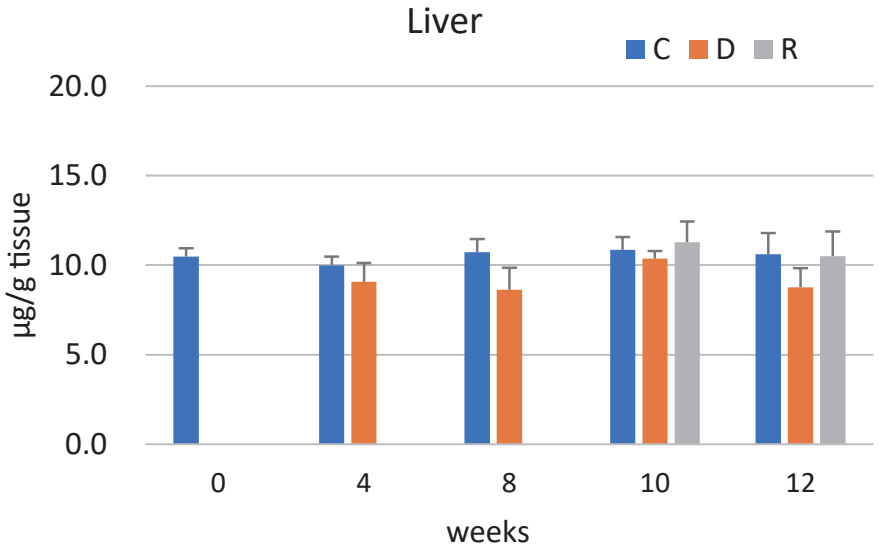

Figure 5: Zinc concentration (mean and S.D. μg/g tissue) in the liver of rats fed zinc deficient diet (D: deficient diet group, R: recovery group, C: control)

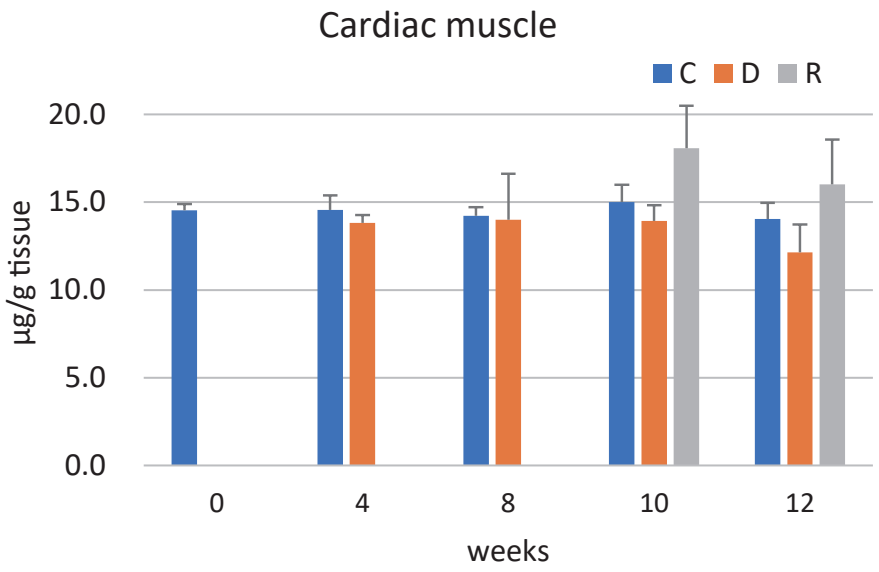

Figure 6: Zinc concentration (mean and S.D. μg/g tissue) in the cardiac muscle of rats fed zinc deficient diet (D: deficient diet group, R: recovery group, C: control)

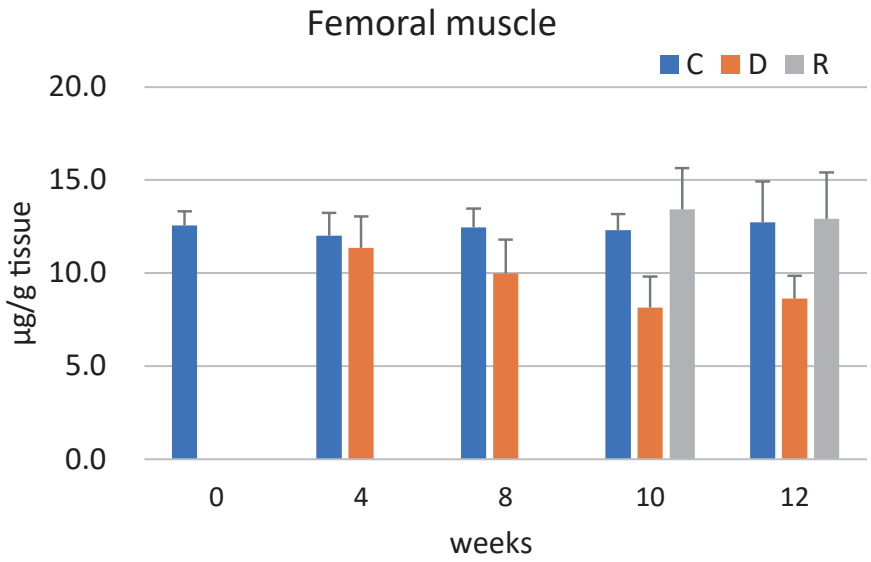

Figure 7: Zinc concentration (mean and S.D. μg/g tissue) in femoral muscle of rats fed zinc deficient diet (D: deficient diet group, R: recovery group, C: control)

Zn Concentration in Liver

Liver tissue Zn concentration in group C fluctuated slightly throughout the experimental period, averaging 10.5 μg/g. Liver tissue Zn concentration in group D decreased to 9.1 μg/g after four weeks and to 8.6 μg/g after eight weeks, but reached 10.4 μg/g, the same level as group C, after 10 weeks, and decreased to 8.8 μg/g after 12 weeks. Liver tissue Zn concentration in R group was 11.3 μg/g, which was higher than that in group C at 10 weeks, two weeks after the change to standard diet, and at 12 weeks, the level was 10.5 μg/g, the same as that in group C.

Zn Concentration in Heart Tissue

Heart tissue Zn concentrations in group C fluctuated slightly and averaged 14.5 μg/g, while those in group D also changed little, but were lower than those in group C, dropping to 12.0 μg/g after 12 weeks. Heart tissue Zn concentrations in group R were significantly higher than those in group C at 18.1 μg/g after 10 weeks and 16.0 μg/g after 12 weeks.

Zn Concentration in Thigh Muscle

Group C had an average thigh muscle Zn concentration of 12.4 μg/g, while Group D showed a significant decrease to 8.6 μg/g at 12 weeks. Thigh muscle Zn concentration in group R was higher than that in group C at 10 weeks, but at the same level as that in group C at 12 weeks. Group D had an average thigh muscle Zn concentration of 12.4 μg/g at 12 weeks, which significantly decreased to 8.6 μg/g at 12 weeks.

PART 2: Distribution of Zn in the Fingertip Blood Samples of Healthy Young Women

To evaluate the efficacy of a method for measuring Zn concentrations using fingertip blood samples that is less invasive than venous blood sampling and has the potential for self-monitoring, fingertip blood and serum Zn concentrations were measured in young women with no health problems.

Methods

Ethics

This study was performed in line with the principles of the Declaration of Helsinki by the approval of the Ethics Committee for Human Research at Sagami Women's University (August 20, 2018; approval no. 18079).

Subjects

The content of the survey was explained to students at a Woman's University in Kanagawa Prefecture, and consent for participation was obtained from 78 of them. The consent form includes the following items: significance and purpose of the research, methods of the research, duration of the research, researchers who will conduct the research, voluntary nature of participation in the research (participation in the research is voluntary and no disadvantage will be suffered by not participating, and that consent can be withdrawn at any time and no disadvantage will be suffered by withdrawing consent), handling of personal information ((maximum consideration shall be given to the protection of the privacy of the subjects), and the handling after the completion of the research and the publication of the research results, with a check box for each item of contact information for inquiries, complaints, etc., and a signature box for consent. Finally, examinations and surveys were conducted with 73 participants (five subjects could not participate the study in September 2018 due to physical condition caused by menstrual cycle, etc.).

Blood Sampling for Zn determination

Blood samples for this study were collected in the morning, because previous studies have shown that serum Zn concentrations tend to fluctuate during the day and decrease in the afternoon.[24] Four participants were unable to have their venous blood drawn. These were due to difficulties in identifying blood vessels and poor physical condition.

Measurement of Fingertip Blood Zn Concentration

Fingers used for blood collection were disinfected with alcohol, punctured with a puncture needle for blood collection (Medisafe needle/Thermo Corporation), and 10 μL blood was collected using a micropipette. The collected blood was added to 990 μL ultrapure water in a microtube and gently mixed. Samples were frozen at –25 ℃ until further analysis. Thawed blood samples were analyzed with high sensitivity via furnace atomization using an AA240 atomic absorption spectrophotometer (Agilent, Australia).

Method for the Determination of Serum Zn Concentration

Forearm was disinfected with alcohol, 5 mL of venous blood was collected from the median cutaneous vein of the forearm, centrifuged, and 1 mL of serum was transferred to acid-washed tubes and stored at –25 ℃ until analysis. Serum Zn levels were measured using atomic absorption spectrophotometry in 69 subjects, excluding those whose blood could not be drawn.

Questionnaire

Participants were asked to fill out the following survey: age, height, weight, health status, change in physical condition in two weeks, lifestyle (frequency of exercise, frequency of alcohol consumption, stress, sleeping hours and time, and method of commuting to school), eating habits (breakfast, lunch, and dinner consumption, frequency of eating out, and frequency of eating in), diet status, use of supplements, and use of Easy Fiber, Five Mini, All Bran, Cream Brown Rice Bran (dietary fiber enriched food, cereal).

Results

As shown in Fig. 8, fingertip blood Zn concentrations averaged 793.5 μg/dL (95% confidence interval [C.I.]: 717.0–870.1 μg/dL). Serum Zn concentrations averaged 76.4 μg/dL (95% C.I.: 78.7–74.2 μg/dL), as shown in Fig. 9. There was no correlation between the serum and whole blood Zn concentrations, as shown in Fig. 10.

Mean Zn concentration in blood components was 1271.6 μg/dL (95% C.I.: 1144.0–1399.1 μg/dL) when calculated with reference to hematocrit values, but as shown in Fig. 11, there were large individual differences in the Zn concentration in blood components regardless of the serum Zn concentration.

Body mass index (BMI) of the subjects ranged from 15.8–28.3, with a mean of 20.5 ± 2.3 (95% C.I.: 19.9–21.1), and the relationship among serum,

whole blood, and calculated blood cell Zn concentrations is shown in Fig. 12. 13 and 14 respectively. Although the differences in serum Zn concentrations between the BMI quartiles were small, Zn concentration tended to be higher in higher BMI quartiles.

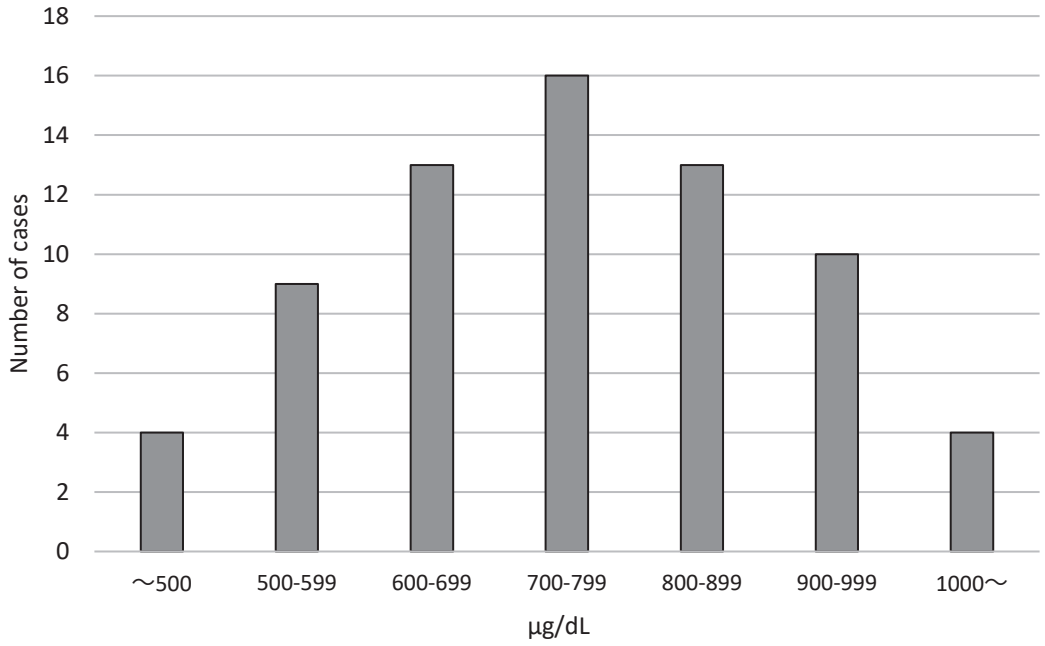

Figure 8: Distribution of zinc concentartion in fingertip blood of young Japanese women

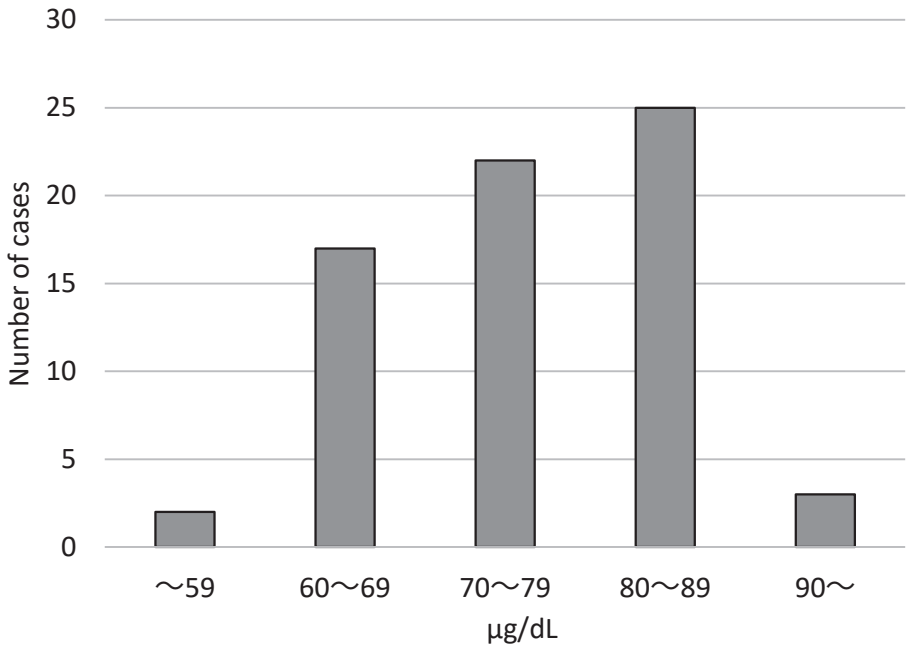

Figure 9: Distribution of serum zinc concentration in young Japanese women

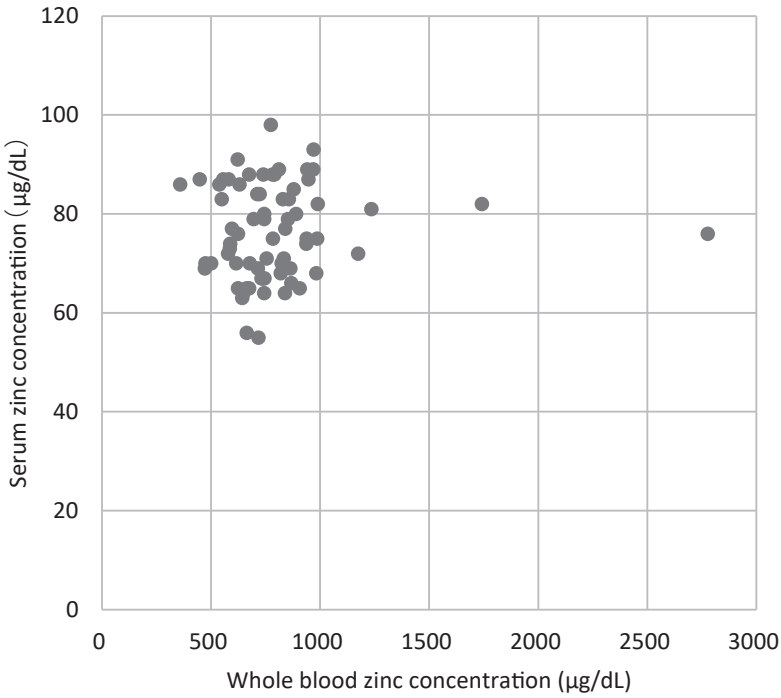

Figure 10: Correlation of zinc concentration between serum and whole blood of young Japanese women (Correlation coefficient R=0.057)

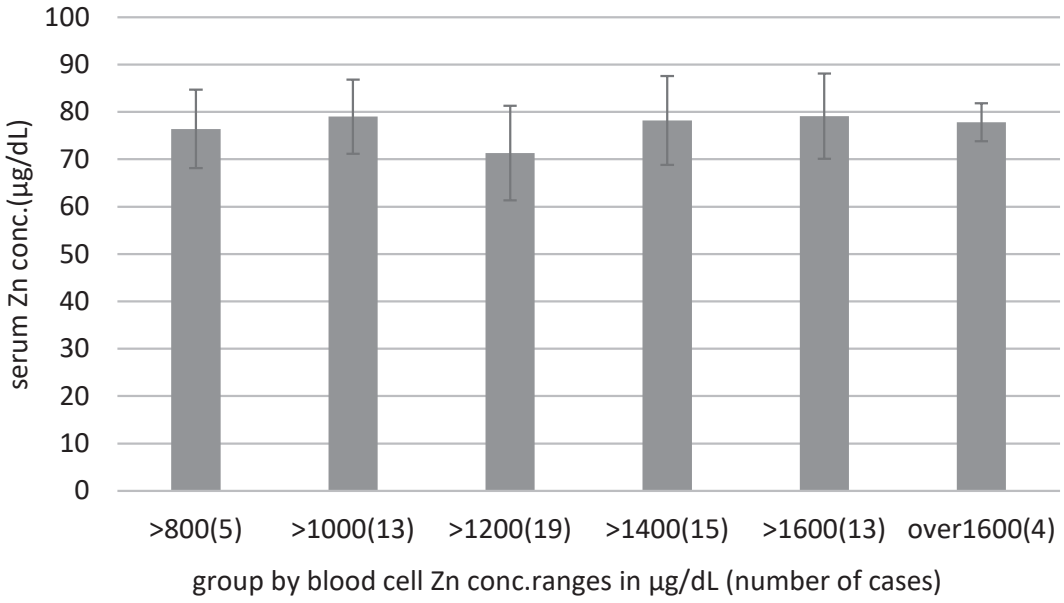

Figure 11: Serum zinc concentration in groups of estimated zinc concentration in blood cell fraction (μg/dL)

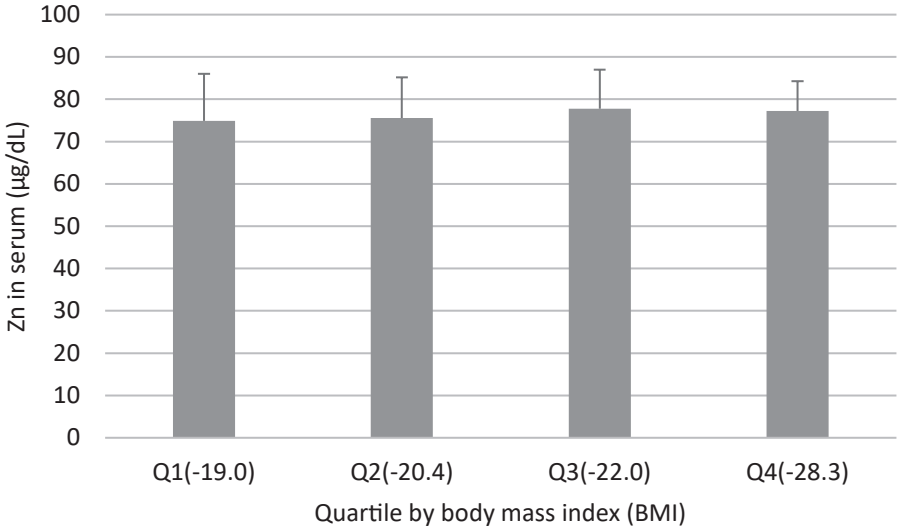

Figure 12: Serum zinc concentration by body mass index (BMI) quartile (μg/dL)

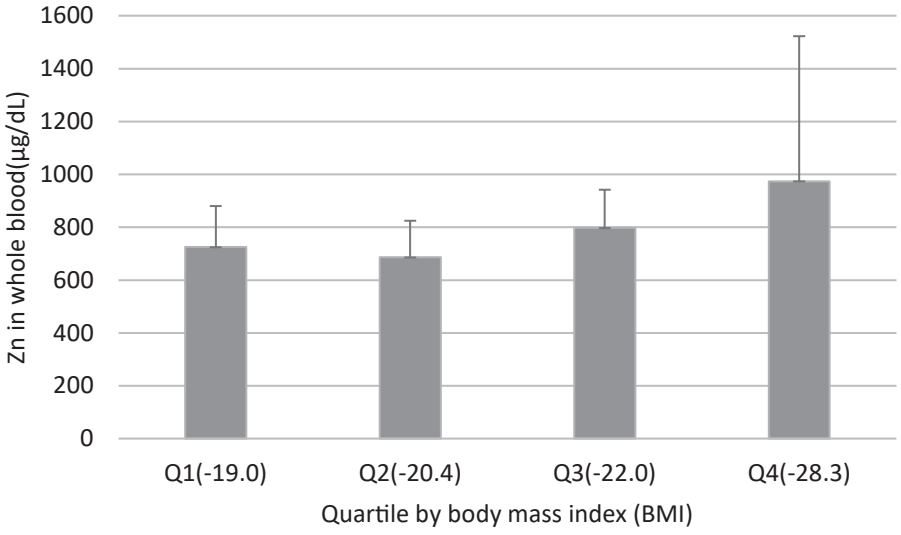

Figure 13: Whole blood zinc concentration by body mass index (BMI) quartile (μg/dL)

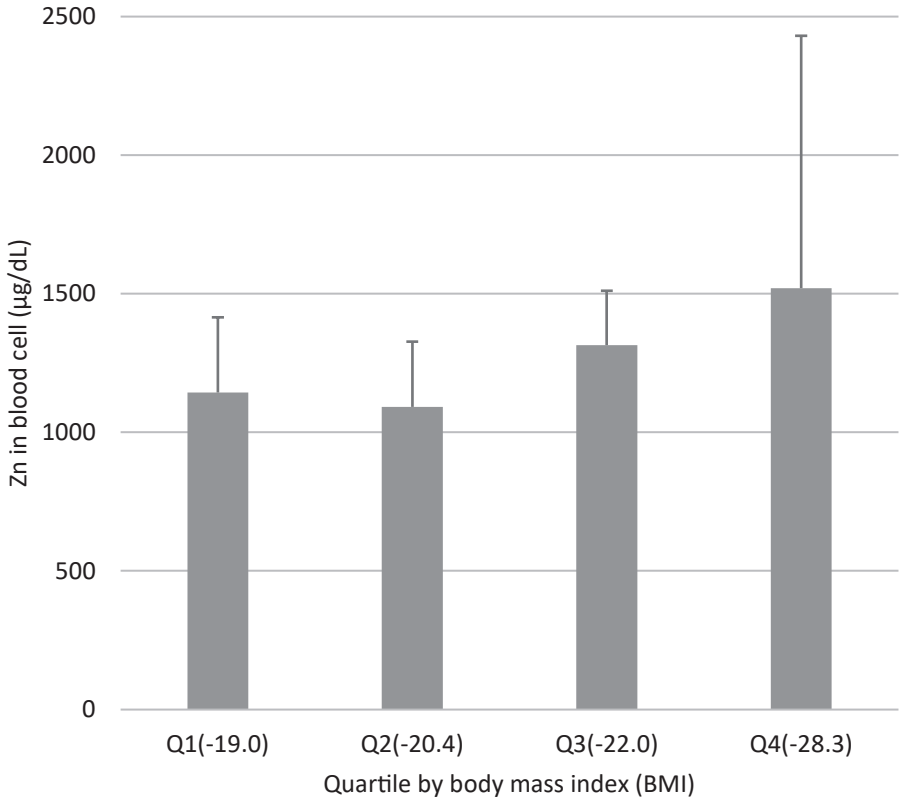

Figure 14: Estimated zinc concentration of blood cell fraction by body mass index (BMI) quartile (μg/dL)

Discussion

Zn is an essential trace element for normal biological functions, as it is involved in more than 300 enzyme activities and in the structural maintenance of more than 1000 proteins.[1, 29] Hunger-related Zn deficiency is one of the most significant nutritional problems in developing countries.[20, 30, 31] However, even in developed countries, due to changes in diet and food, daily intake of Zn relative to requirements has been reported to be inadequate, indicating that Zn deficiency or sub-deficiency states are globally widespread.[21, 32, 33, 34] The National Health and Nutrition Examination Survey (2019) reported that both men and women in their 20s and above did not reach the recommended amounts set by the Dietary Reference Intakes for Japanese population, 2020 Edition.[12] In a previous study, the authors examined the impact of dietary information provision on improving low Zn levels in young women. [35] That study revealed that approximately 60% of the subjects were potentially Zn-deficient before the provision of dietary information. Therefore, the prevalence of Zn deficiency in Japan is a concern not only among the children and elderly, but also among other age groups, including young people.

Increase in emaciation among young women in Japan has become a serious issue. As per the National Health and Nutrition Survey, the percentage of thin women (BMI: <18.5 kg/m2) was 11.8% in 2019. It was particularly high among women aged 15–29 years (21%).[36] This may be due to restricted dietary intake for the purpose of weight loss because of the desire to be thin among young women, and restricted dietary intake

without nutritional considerations is suggested to be associated with Zn deficiency status.[37, 38, 39]

Serum or plasma Zn concentration is a common diagnostic criterion for Zn deficiency, and the efficacy of Zn drug administration in treatment is assessed based on serum or plasma Zn concentration.[14, 20] In our previous report, we observed that Zn supplementation increased the Zn concentration regardless of the pre-administration of Zn.[43] This phenomenon has been observed in many other studies, where external Zn concentrations were reflected in serum Zn concentrations.[40] However, in relation to clinical Zn deficiency, the sensitivity and specificity of serum or plasma Zn concentrations are not high, and the frequency of false-negatives or -positives is also high.[5, 16, 18]

In this study, we examined the efficacy of quantifying trace amounts of Zn in whole blood as an indicator of Zn deficiency, which is not a common health examination item. To obtain a trace amount of whole blood, fingertip blood was used as the sample and a frameless atomic absorption method was employed, which allowed the rapid analysis of diluted hemolyzed samples without any pretreatment. To evaluate the validity of the analytical method and determine whether the analytical values reflect the Zn sufficiency in the body, we conducted animal experiments and took actual measurements from a human population.

Severe deficiency may be caused by Zn restriction in growing rats. Serum Zn concentrations were markedly reduced, but there was no significant difference in the tissue Zn concentrations in the control group. During acute deficiency due to Zn restriction, serum Zn levels were also markedly reduced, but liver and intramyocardial Zn concentrations remained constant, with only a significant decrease observed in the skeletal muscle, likely reflecting the reduced weight gain and efficient utilization of Zn stores in the body.[5, 41] When the blood Zn concentration was calculated from the serum Zn concentration and hematocrit value with the Zn concentration in the tail vein blood as the whole blood Zn concentration, there was no decrease in the blood Zn concentration in the deficient group as shown in the Fig. 4, and on the contrary, it was slightly higher than that in the control group. In an animal experiment, Zn deficiency was induced by rearing growing rats on a Zn-deficient diet, followed by Zn recovery by returning them to a normal diet. In this experiment, plasma Zn concentrations were observed to fluctuate quickly, reflecting the Zn intake, and leukocyte counts were observed to significantly increase by more than 2-fold three weeks after the initiation of treatment.[42] The slight increase in the calculated whole blood Zn concentration in this study (Fig. 4) may be due to the increased leukocyte count. Zn concentration in leukocytes is higher than that in erythrocytes and could be used as an indicator of Zn deficiency.[5] However, separation of blood cell components requires at least 10 mL of blood and complex laboratory manipulations.

Serum Zn concentrations in the young female subjects of this study were similar to those reported in previous literature, and we considered them to be included in the category of healthy young Japanese women.[12] Our previous study was on a similar population, and 61% of the subjects in that study and 59% of the subjects in the present study were determined to be Zn-deficient with less than 80 μg/dL Zn, the threshold level proposed by the Japan Society for Biomedical Research on Trace Elements.

Fingertip blood Zn concentration in this study was determined to be 793.5 μg/dL (95% C.I.: 717.0–870.1 μg/dL), slightly higher than the reported venous blood Zn concentration.[43, 44] Peripheral blood is reported to have higher erythrocyte and white blood cell counts than venous blood, which may be the cause of higher fingertip blood Zn concentrations.[45] Fingertip blood Zn concentration did not correlate with the serum Zn concentration. Blood cellular component Zn concentration calculated from the fingertip blood and serum Zn concentrations with reference to the hematocrit value was found to be 1271.6 μg/dL (95% C.I.: 1144.0–1399.1 μg/dL), while the serum Zn concentration by blood cell Zn concentration

ranged from 71.3–79.1 μg/dL. Individual differences in the fingertip blood Zn concentration were considered to be determined by the blood cell Zn concentrations.

Regarding the relationship between Zn concentration and body size, a meta-analysis of the relationship between obesity/overweight and serum Zn concentrations reported significantly lower serum Zn concentrations in obese individuals in both children and adults.[46] However, the present study found no relationship between serum Zn levels and BMI. This may be because only a small number of subjects in this study were obese and many tended to be thin. However, whole blood and blood cell Zn concentrations tended to be higher in those at high BMI quartiles.

In Zn deficiency experiments in rats, serum Zn concentrations decreased rapidly, but there was no significant difference in the converted blood Zn concentrations in the deficient group or in the group recovering after deficiency compared to the control group; rather, they tended to be higher during deficiency. When blood cell Zn concentrations were similarly determined in humans, there was no relationship with serum Zn concentrations, but large individual differences. Analysis of Zn concentrations in isolated blood cell components revealed that lymphocytes and leukocytes contained 4–7 times more intracellular Zn than erythrocytes. Platelets contain approximately half the number of erythrocytes.[47, 48] Given the normal ratio of blood cell components, low leukocyte and lymphocyte counts are largely due to the Zn content in red blood cells. However, it is difficult to capture the changes in erythrocyte Zn concentration because of its slow turnover, and some reports suggest that neutrophil Zn concentration or Zn metabolic capacity is more appropriate for determining the Zn sufficiency status in the body.[49]

Flameless atomic absorption techniques have long been used for Zn analysis of blood materials, and their application to Zn analysis of blood samples has been studied for many years.[4, 36, 50] Although inductively coupled plasma (ICP) and ICP-mass spectrometry (MS) have also been applied to the determination of Zn in biological samples as a result of recent advances, the ability to analyze trace samples without any pretreatment, such as acid digestion of organic components, is unique to flameless atomic absorption spectrometry. However, the ability to analyze trace samples without any pretreatment is unique to flameless atomic absorption spectrometry. For fingertip blood, it is suitable to mix the blood with ultrapure water after collection and analyze it on the same day; however, there is no degradation even if it is refrigerated and analyzed within a few days. Refrigeration is undesirable because of the presence of denatured proteins, but the addition of a surfactant or other agent may facilitate stable analysis. With the analysis protocol used in this study, the analysis of 50 samples can be completed in less than 2 h, and due to the simplicity of the blood collection method, it can be used for mass screening and public health studies. Another advantage is that the price of the instrument is approximately half to one-fifth of that of ICP and ICP-MS for frameless atomic absorption spectrometry.

One limitation of this study is that the subjects analyzed were young women with no health problems, so data are not available for men, other age groups, or individuals with symptoms specific to Zn deficiency, such as taste disorders. To establish a screening level for identifying patients with Zn deficiency, it is necessary to examine the relationship between the amount of Zn in the body exchangeable zinc pool and fingertip blood. In the present study, 2 of the 60 subjects had markedly elevated levels of Zn, but they were in good health, and their Zn intake did not differ from that of the general population. Red blood cell, white blood cell, and lymphocyte counts were not measured. The differences in Zn concentrations among these blood cells did not allow us to examine how the composition of blood cells reflected these differences.

In conclusion, the method of measuring the Zn concentration in whole blood using trace amounts of fingertip blood is widely feasible as a simple

method of evaluation. Biochemical and clinical analyses of the causes of these significant individual differences in Zn concentrations in blood cells are needed. We believe that this method shows high public health value and can be used as a new screening method for assessing Zn sufficiency after further validation.

References

1. Prasad AS (2013) Discovery of Human Zinc deficiency: its impact on human health and disease. Adv Nutr 4:176–190 DOI: 10.3945/an.112.003210
2. Lin PH, Sermersheim Ma, Li H, Lee PHU, Steinberg SM, Ma J (2018) Zinc in Wound Healing Modulation. Nutrients 10:16. doi:10.3390/nu10010016
3. Yan Wan, Bingkun Zhang (2022) The impact of zinc and zinc homeostasis on the intestinal mucosal barrier and intestinal diseases. Biomolecules 12:900 doi: 10.3390/biom12070900.
4. Stevens MD, MacKenzie WF, Anand VD (1977) A simplified method for determination of zinc in whole blood, plasma, and erythrocytes by atomic absorption spectrophotometry. Biochem Med 18:158-63.
5. Goode HF, Kelleher J, Walker BE (1989) Zinc concentrations in pure populations of peripheral blood neutrophils, lymphocytes and monocytes. Ann Clin Biochem 26:89-95. doi: 10.1177/000456328902600114.
6. Hambidge M (2003) Biomarkers of trace mineral intake and status J Nutr 133: 948S–955S. https://doi.org/10.1093/jn/133.3.948S
7. Akanli L, Lowenthal DB, Gjonaj S, Dozor AJ (2003) Plasma and red blood cell zinc in cystic fibrosis. Pediatr Pulmonol 35:2-7 DOI: 10.1002/ppul.10195
8. Lowe NM, Woodhouse LR, Sutherland B, Shames DM, Burri BJ, Abrams SA, Turnlund JR, Jackson MJ, King JC (2004) Kinetic parameters and plasma zinc concentration correlate well with net loss and gain of zinc from men. J Nutr 134:2178-2181 doi: 10.1093/jn/134.9.2178.
9. Gibson RS, Hess SY, Hotz C, Brown KH (2008) Indicators of zinc status at the population level: a review of the evidence. Br J Nutr 99, Suppl. 3, S14–S23 doi: 10.1017/S0007114508006818.
10. Miller LV, Hambidge KM, King JC, Westcott JE, Krebs NF (2017) Predictors of the size of the exchangeable zinc pool differ between children and adults. J Nutr 147:187–94. https://doi.org/10.3945/jn.116.239541
11. Tanvir EM, Whitfield KM, Ng JC, Shaw PN (2020) Development and validation of an ICP-MS method and its application to determine multiple trace elements in small volumes of whole blood and plasma. J Anal Toxicol 44:1036–1046 doi: 10.1093/jat/bkaa033
12. Yokokawa H, Fukuda H, Saita M, Miyagami T, Takahashi Y, Hisaoka T, Naito T (2020) Serum zinc concentrations and characteristics of zinc deficiency/marginal deficiency among Japanese subjects. J Gen Fam Med 21(6):248-255. doi: 10.1002/jgf2.377.
13. Hennigar SR, Lieberman HR, Fulgoni III VL, McClung JP (2018) Serum zinc concentrations in the US population are related to sex, age, and time of blood draw but not dietary or supplemental zinc. J Nutr 148:1341–135. DOI: 10.1093/jn/nxy105
14. Kodama H, Tanaka M, Naito Y, Katayama K, Moriyama M (2020) Japan's practical guidelines for zinc deficiency with a particular focus on taste disorders, inflammatory bowel disease, and liver cirrhosis. Int J Mol Sci 21:2941. doi:10.3390/ijms21082941
15. Hennigar SR, Kelley AM, Anderson BJ, Armstrong NJ, McClung HL, Berryman CE, Karl JP, McClung JP (2021) Sensitivity and reliability of zinc transporter and metallothionein gene expression in peripheral blood mononuclear cells as indicators of zinc status: responses to ex vivo zinc exposure and habitual zinc intake in humans. Br J Nutr 125: 361–368. doi: 10.1017/S0007114520002810.
16. Wood RJ (2000) Assessment of marginal zinc status in humans. J Nutr 130(5S Suppl):1350S-

4S. DOI: 10.1093/jn/130.5.1350S
17. King JC (2011) Zinc: an essential but elusive nutrient Am J Clin Nutr 94:679S–684S https://doi.org/10.3945/ajcn.110.005744
18. Moran VH, Stammers AL, Medina MW, Patel S, Dykes F, Souverein OW, Dullemeijer C, Pérez-Rodrigo C, Serra-Majem L, Nissensohn M, Lowe NM (2012) The relationship between zinc intake and serum/plasma zinc concentration in children: A systematic review and dose-response meta-analysis. Nutrients 4:841-858. doi:10.3390/nu4080841
19. Wieringa FT, Dijkhuizen MA, Fiorentino M, Laillou A, Berger J (2015) Determination of zinc status in humans: which indicator should we use? Nutrients 7, 3252-3263 doi:10.3390/nu7053252
20. Secretariat on behalf of the participants: de Benoist B, Darnton-Hill I, Davidsson L, Fontaine O, Hotz C (2007) Conclusions of the joint WHO/UNICEF/IAEA/IZiNCG interagency meeting on zinc status indicators. Food Nutr Bull 28: suppl doi: 10.1177/15648265070283S306.
21. Kvamme JM, Grønli O, Jacobsen BK, Florholmen J (2014) Risk of malnutrition and zinc deficiency in community-living elderly men and women: the Tromsø Study. Public Health Nutr 18: 1907–1913. doi:10.1017/S1368980014002420
22. Kogirima M, Ohta N, Kubo A, Komatsu M, Watanabe E (2017) Nutritional assessment of zinc using the data of National Health and Nutrition Survey in Japan from 1946 to 2015. (in Japanese) Trace Nutrients Research 34:102-108
23. Lien EL, Boyle FG, Wrenn JM, Perry RW, Thompson CA, Borzelleca JF (2001) Comparison of AIN-76A and AIN-93G diets: a 13-week study in rats. Food Chem Toxicol 39:385-92. DOI: 10.1016/s0278-6915(00)00142-3
24. Masuda T, Yamamoto A, Toyohara H (2015) The iron content and ferritin contribution in fresh, dried, and toasted nori, *Pyropia yezoensis*. Biosci Biotechnol Biochem 79:74–81 https://doi.org/10.1080/09168451.2014.968087
25. Wang W, Matsushita Y, Aoki D, Fukushima K, Tomioka R, Iizuka K, Takenaka C (2017) Decontamination of Cs from Japanese cedar (*Cryptomeria japonica*) via kraft cooking. Journal of Wood Science 63:388–395. https://doi.org/10.1007/s10086-017-1628-1
26. Kakinuma M, Yasumoto K, Suzuki M, Kasugai C, Koide M, Mitani K, Shidoji K, Kinoshita S, Hattori F, Maeyama K, Awaji M, Nagai K, Watabe S (2020) Trivalent iron is responsible for the yellow color development in the nacre of akoya pearl oyster shells. Mar Biotechnol 22:19–30. https://doi.org/10.1007/s10126-019-09927-5
27. Matsumoto S, Doi H, Kasuga J (2022) Changes over the years in soil chemical properties associated with the cultivation of ginseng (Panax ginseng Meyer) on andosol soil. Agriculture 12:1223. https://doi.org/10.3390/agriculture12081223
28. Iizuka A, Adachi K, Shibata E (2022) Investigation of the scorodite formation mechanism in As(V) solution containing Fe(II) with hematite addition using a stable iron isotope. Mater Trans 63:655-661. https://doi.org/10.2320/matertrans.M-M2022801
29. Hara T, Takeda T, Takagishi T, Fukue K, Kambe T, Fukada T (2017) Physiological roles of zinc transporters: molecular and genetic importance in zinc homeostasis. J Physiol Sci 67:283–301 DOI 10.1007/s12576-017-0521-4
30. Wessells KR, Brown KH (2012) Estimating the global prevalence of zinc deficiency: Results based on zinc availability in national food supplies and the prevalence of stunting. PLoS ONE 7:e50567. https://doi.org/10.1371/journal.pone.0050568
31. Tran CD, Gopalsamy GL, Mortimer EK, Young GP (2015) The potential for zinc stable isotope techniques and modelling to determine optimal zinc supplementation. Nutrients 7:4271-4295. doi:10.3390/nu7064271
32. Lönnerdal B (2000) Dietary factors influencing zinc absorption. J Nutr 130:13078S-1383S. doi:10.1093/jn/130.5.1378S.
33. Cecília R, Falcão TMA, Lyra CO, Morais

CMM, Pinheiro LGB, Pedrosa LFC, Lima SCVC, Sena-Evangelista KCM (2019) Processed and ultra-processed foods are associated with high prevalence of inadequate selenium intake and low prevalence of vitamin B1 and zinc inadequacy in adolescents from public schools in an urban area of northeastern Brazil. PLoS One 14:e0224984. https://doi.org/10.1371/journal.pone.0224984

34. Zhang Y, Liu Y, Qiu H (2018) Association between dietary zinc intake and hyperuricemia among adults in the United States. Nutrients 10:568. doi:10.3390/nu10050568
35. Han S, Mizoi M, Nakatani E, Adachi S, Miyakoshi Y, Yanagisawa H (2015) Improvement of serum zinc levels in young Japanese women by provision of food information. Biol Trace Elem Res 164:169-177. DOI 10.1007/s12011-014-0211-5
36. Helth Service Division, Ministry of Health, Labour and Welfare, Japan (2019) National Health and Nutrition Survey (NHNS) in Japanese. https://www.mhlw.go.jp/content/10900000/000687163.pdf
37. Hayashi F, Takimoto H, Yoshita K, Yoshiike N (2006) Perceived body size and desire for thinness of young Japanese women: a population-based survey. Br J Nutr 96:1154-62. doi: 10.1017/bjn20061921.
38. Yoshinaga M, et al (2019) Generational survey of blood zinc concentrations in patients undergoing medical examinations at our hospital (in Japanese). Sogou Shinryo 46:273-275 https://doi.org/10.7143/jhep.46.273
39. Yachi Y (2019) Overweight and its harmful effects in young Japanese women and pregnant women (in Japanese). New Diet Therapy 34,27-32
40. Katayama K, Hosui A, Sakai Y, et al (2020) Effects of zinc acetate on serum zinc concentrations in chronic liver diseases: a multicenter, double-blind, randomized, placebo-controlled trial and a dose adjustment trial. Biol Trace Elem Res 195:71–81. https://doi.org/10.1007/s12011-019-01851-y
41. Ogawa Y, Kinoshita M, Shimada S, Kawamura T (2018) Zinc and skin disorders. Nutrients 10:199. doi:10.3390/nu10020199
42. Sakakibara Y, Sato S, Kawashima Y, Someya Y, Shirato K, Tachiyashiki K, Imaizumi K (2011) Different recovery responses from dietary zinc-deficiency in the distribution of rat granulocytes. J Nutr Sci Vitaminol (Tokyo) 57:197-201. DOI: 10.3177/jnsv.57.197
43. Poddalgoda D, Macey K, Hancock S (2019) Derivation of biomonitoring equivalents (BE values) for zinc. Regul Toxicol Pharmacol 106:178–186. doi: 10.1016/j.yrtph.2019.04.018.
44. Zeng HL, Li H, Lu J, Guan Q, Cheng L (2019) Assessment of 12 metals and metalloids in blood of general populations living in Wuhan of China by ICP-MS. Biol Trace Elem Res 189:344–353. https://doi.org/10.1007/s12011-018-1486-8
45. Kitano M, Morita M (1960) An observation on the difference between specific garavities of venous and peripheral blood. Journal of the Japan Society of Blood Transfusion 7:129-139.
46. Gu K, Xiang W, Zhang Y, Sun K, Jiang X (2019) The association between serum zinc level and overweight/obesity: a meta-analysis. Eur J Nutr 58:2971–2982. doi: 10.1007/s00394-018-1876-x.
47. Whitehouse RC, Prasad AS, Rabbani PI, Cossack ZT (1982) Zinc in plasma, neutrophils, lymphocytes, and erythrocytes as determined by flameless atomic absorption spectrophotometry. Clin Chem 28(3):475-80. https://doi.org/10.1093/clinchem/28.3.475
48. Milne DB, Ralston NV, Wallwork JC (1985) Zinc content of cellular components of blood: methods for cell separation and analysis evaluated. Clin Chem 31:65-9. https://doi.org/10.1093/clinchem/31.1.65
49. Prasad AS (1985) Laboratory diagnosis of zinc deficiency. J Am Coll Nutr 4:591-8. doi: 10.1080/07315724.1985.10720101.
50. Vieira NE, Hansen JW (1981) Zinc determined

in 10-microL serum or urine samples by flameless atomic absorption spectrometry. Clin Chem 27:73-7.

Statements and Declarations

Funding

This study was supported by the grant in aid for Selected Research B of Sagami Women's University (2017).

Competing interests

The authors declare no competing interests.

Author Contributions

All authors contributed to the study conception Material preparation, Data collection, Analysis and interpretation of data were completed by Yuka Miyajima. Original first draft was written by Yuka Miyajima. All authors read and approved the final manuscript. This study was conducted as YM's doctoral dissertation.

Data Availability

The authors state the availability of necessary data from the corresponding author upon reasonable request.

Ethics Approval

Ethics Approval The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Animal experiment part was approved by the Research Ethics Committee of the Sagami Women's University Experimental Animal Committee (April 20, 2017; approval no. 2017-02). Human volunteer survey part was conducted in line with the Helsinki Declaration and approved by the Ethics Committee for Human Research at Sagami Women's University (August 20, 2018; approval no. 18079).