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Short Communication

Iron and zinc deficiency affect adolescent school girls' behavior

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Abstract

Background: Iron and zinc are important trace elements in humans. Deficiencies in these elements have been reported in several studies. However, few studies have investigated blood examination results in conjunction with human behavior. Thus, this study aimed to analyze students' exercise, daily habits, and behavior in combination with blood laboratory examinations over a two-year study period.

Methods: Healthy female junior high and high school students were recruited between 2020 and 2021. Body weight and height were measured during annual physical and blood examinations. A survey was conducted on the type and frequency of exercise, and daily exercise habits. The data obtained were analyzed using a *t*-test, and logistic analysis was performed to compare quantitative characteristics.

Results: A total of 102 female students participated in this study. Among them, 51 students were examined twice over two consecutive years. The prevalence of iron, asymptomatic zinc (60–79 μg/dL), and symptomatic zinc (<60 μg/dL) deficiencies was 7.8, 47.6, and 1.2%, respectively. Only one student had symptomatic zinc deficiency based on the serum zinc concentration. This participant reported having rough skin and prolonged injury healing. Serum iron concentrations were significantly higher in the extreme exercise group than in the non-exercise group. No significant correlation was observed between serum zinc concentration and exercise habits.

Conclusions: Adolescent girls who exercise regularly may be motivated to maintain their iron intake. Serum zinc concentrations may affect skin-disordered behaviors in adolescent girls.

Keywords: Zinc, Iron deficiency, Trace elements, Adolescents, School

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Introduction

Minerals play an important role in the human body [1]. Iron is an essential element in humans, and its deficiency causes anemia in infants [2] and adolescents [3]. Besides anemia, iron deficiency causes restless legs syndrome, pica, fatigue, and cognitive dysfunction, which may result in the degradation of quality of life [4,5]. Although various studies have evaluated iron deficiency in European adolescents, reports on iron deficiency in Japanese adolescents are limited. For example, Ueda et al. reported that iron deficiency anemia is common in adolescent athletes [6].

Zinc is an essential element that catalyzes more than 300 enzymatic reactions [1]. It is vital for growth and development and is important in wound healing, taste, and smell [7]. Compared with iron, the need for zinc might be underestimated because physical symptoms are not fully recognized in daily life, especially among adolescents [8]. In addition, few studies have objectively evaluated feelings regarding lifestyle and trace elements. Following the practice guidelines for zinc deficiency of the Japanese Society of Clinical Nutrition [9], asymptomatic zinc deficiency was defined as a serum zinc concentration of 60–80 μg/dL, whereas symptomatic zinc deficiency was defined as a serum zinc concentration of <60 μg/dL. The correlation between serum zinc concentration and physical symptoms remains unclear. Ohguri et al. reported that adult women who exercised daily had higher serum zinc concentrations than those who did not exercise [10].

Thus, this study aimed to examine the effects of iron and zinc as trace materials on the behavior of adolescent girls in junior high and high school. This was achieved by surveying students' daily habits and collecting blood examination data over two years.

Methods

Participants

Healthy female students aged 12–18 years were recruited from a school in Tokyo, Japan, between 2020 and 2021. Student examinations and survey data were obtained consecutively over two years.

This study was approved by the Ethics Committee of the Juntendo University Nerima Hospital (approval no. N20-0013). Written informed consent was obtained from all the students before their participation.

Physical and blood examinations

The school conducts annual health check-ups, including physical and blood examinations. Physical examination data, including body weight and height, were used to calculate the body mass index (BMI)

Table 1 Examination summary.

	Average	Minimum	Maximum	SD
Physical examination (n = 153 examinations)				
Age, year	15.6	12.1	17.9	1.435
Height, cm	159.0	142.2	175.4	5.982
Weight, kg	52.5	36.1	75.3	6.790
BMI	20.8	15.7	30.6	2.428
Height SD	0.4	−1.8	3.5	1.096
Weight SD	0.1	−1.9	2.9	0.830
Laboratory data (n = 153 tests)				
Hb (g/dL) [11.6–14.9]	13.6	8.7	15.9	1.177
RBC ($\times 10^4$/μL) [390–510]	465	392	541	31.67
AST (IU/L) [12.0–29.5]	19.6	11	37	5.207
ALT (IU/L) [9.0–30.5]	12.7	5	56	6.408
Total cholesterol (mg/dL) [125–230]	187.5	125	301	28.50
Iron (μg/dL) [22–177]	101.3	17	273	40.45
Zinc (μg/dL) [85–117]	84.1	58	118	12.05

SD: Standard Deviation. Height and weight SD represent the standard deviation of Japanese standard body proportion in 2000. Values in parentheses indicate the normal range in the adolescent female population [11].

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and standard deviation (SD). Standard height and weight were defined according to the 2000 Statistical Report [11]. Blood samples were collected in a non-fasted state without consideration of the menstrual cycle. Various parameters, including blood cell count, general biochemical parameters, and serum iron and zinc concentrations were measured (Table 1). The abnormal values of iron and zinc were defined based on previous literature [9,11].

Survey

Questionnaires containing items on exercise type, frequency, and daily habits were distributed to participants (see supplementary document). The questionnaire comprised multiple-choice items that allowed participants to freely provide additional responses with extra choices. For exercise habits, if students indicated participation in ≥2 sports activities, the toughest sports activity was chosen. For example, volleyball was selected if the answers were walking or volleyball. Some students returned the form in both of the years. Regarding the activities of daily living, the answer choices were negative, neutral, and positive. Negative and neutral answers were considered negative, whereas positive responses were considered positive for t-test evaluation. Only the questionnaire from the first year was used because it was unclear which answer should be used in cases where the responses differed between 2 years.

Statistical analysis

A minimal sample size of 50, with 10 and 0.95 for sample differentiation and power (1-β error probability), respectively, was assumed. The quantitative characteristics of the individuals were compared using a t-test or chi-square test. A paired t-test was applied, and logistic analysis was performed to obtain a Receiver Operating Characteristic (ROC) curve. Statistical analyses were performed using JMP software for Windows version 14.3.0 (SAS Institute Inc., Cary, NC, USA).

Results

A total of 102 female students were enrolled in the study, accounting for 41.5% of the total student population. In addition, 51 students underwent two examinations. Table 1 presents the results of the physical and laboratory examinations. The participants exhibited no noticeable differences in body state compared to the normal Japanese population, as indicated by the SD being close to 1.0. The descriptive statistics of the blood parameters were within normal ranges. No significant correlations were observed between physical examination and laboratory data. The SD of serum iron concentration was highest in our analyses, as described

Table 2 Prevalence of iron and zinc deficiency.

		Iron		
		Normal	Deficiency	Total
Zinc	Normal	86	7	93
	Asymptomatic deficiency	54	5	59
	Symptomatic deficiency	1	0	1
	Total	141	12	153

Iron deficiency was described as less than 48 μg/dL. Asymptomatic zinc deficiency was described as 60–79 μg/dL. Symptomatic zinc deficiency was described as less than 60 μg/dL.

Table 3 Survey responses for exercise habits.

	None	Normal	Extremely	Total
Walking	3	4	0	7
Running	0	2	0	2
Skating	0	2	1	3
Dance	4	5	1	10
Tennis	1	1	0	2
Basketball	0	2	0	2
Badminton	3	1	0	4
Volleyball	1	6	17	24
Yoga	4	3	0	7
Roller skating	0	1	0	1
Kendo	0	3	0	3
Swimming	1	0	0	1
Skipping	1	0	0	1
Others	0	1	0	1
None	14	0	0	14

in a previous study [15].

Serum iron and zinc concentrations are shown in Table 2. The cutoff value for iron was 48 μg/dL [11], whereas the cutoff value for zinc was 60–79 and <60 μg/dL for asymptomatic and symptomatic zinc deficiency, respectively. In our cohort, the iron deficiency, asymptomatic zinc deficiency, and symptomatic zinc deficiency rates were 7.8, 47.6, and 1.2%, respectively. The correlation between serum iron and zinc concentrations from 153 examinations is shown in Fig. 1. No significant correlation was observed between the serum iron and zinc concentrations. Exercise habits, including sport type and strength, were evaluated simultaneously, and no correlations were found between theses and any of the blood test results.

The results of the questionnaire survey on the students' exercise habits are shown in Table 3. The answers included sports activities inside (i.e., club membership) and outside school. The number of students with volleyball and dance habits was greater than the number of students with other exercise habits, indicating that these clubs had many members. Volleyball players exhibited the highest exercise intensity. Notably, one student did not participate in any sports activities.

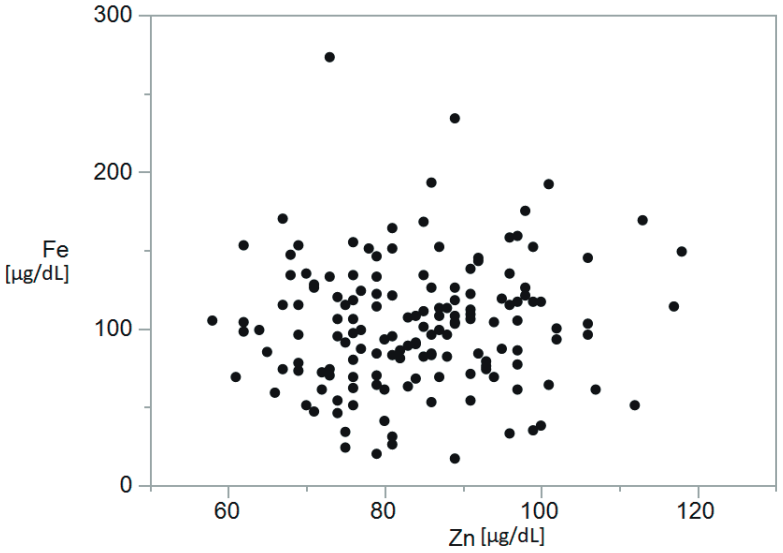

Fig. 1 Correlation between serum iron and zinc concentrations from 153 blood examinations. No significant correlation was observed.

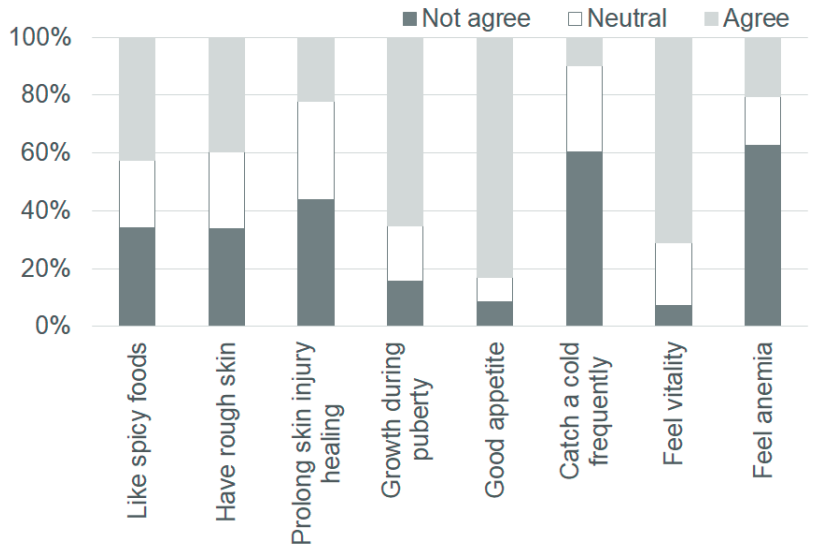

Fig. 2 Results of the survey on daily living activities. The distribution of responses varied across questions, with no significant differences observed between survey results regarding habits or exercise.

Table 4 Survey response for daily living parameters combined with laboratory data.

(a) Iron deficiency

	Not agree		Neutral		Agree	
	Nor	Def	Nor	Def	Nor	Def
Feel vitality	5	1	15	2	55	3
Feel anemia	49	2	12	1	14	3

(b) Zinc deficiency

	Not agree			Neutral			Agree		
	Nor	Asy	Sym	Nor	Asy	Sym	Nor	Asy	Sym
Like spicy foods	14	14	0	9	9	1	18	16	0
Have rough skin	16	11	0	12	10	0	13	18	1
Prolong skin injury healing	24	12	0	9	18	0	8	9	1
Growth during puberty	9	4	0	7	7	1	25	28	0
Good appetite	3	3	0	3	4	0	35	32	1
Catch a cold frequently	26	24	0	11	11	1	4	4	0
Feel vitality	3	2	1	13	4	0	25	33	0
Feel anemia	24	27	0	8	5	0	9	7	1

Numbers in each cell represent the number of participants. Nor: normal. Def: iron deficiency. Asy: asymptomatic zinc deficiency. Sym: symptomatic zinc deficiency.

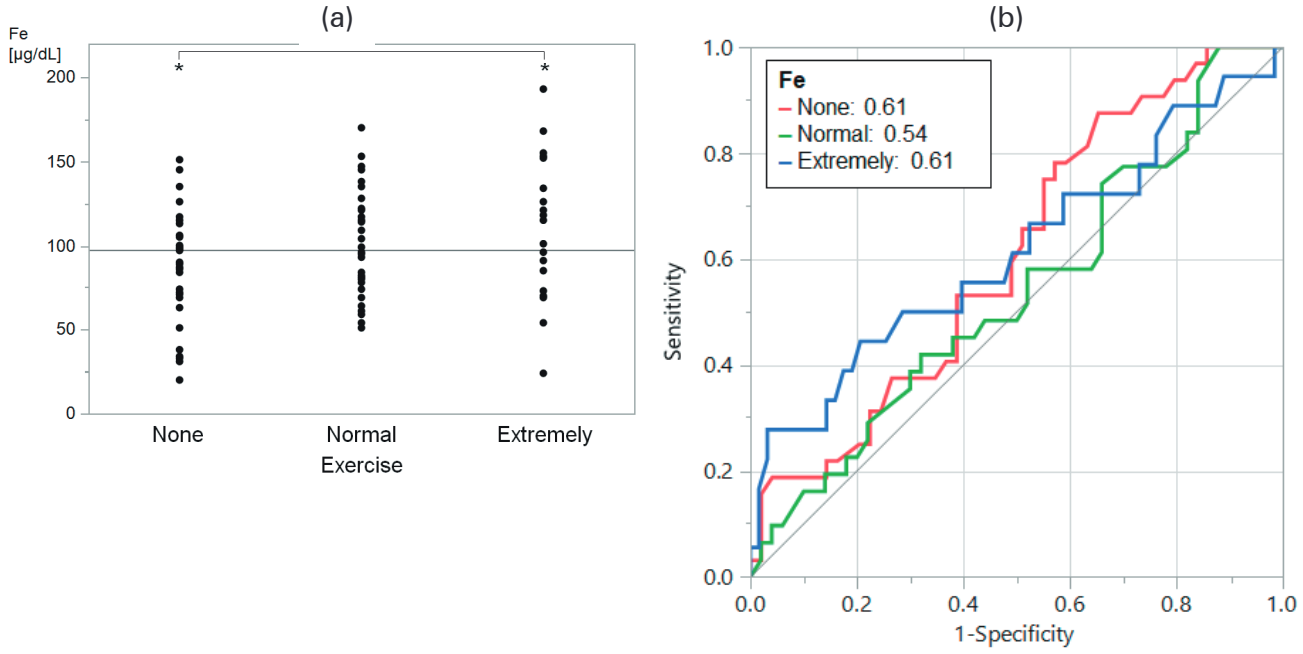

Fig. 3 (a) Univariate analysis of iron concentration and exercise habits. Asterisk (*) indicates a significant difference with $p = 0.0313$. The horizontal line shows the average of the whole data, 97.4 μg/dL. (b) Receiver Operating Characteristic (ROC) curve of iron concentration and exercise habits. The numbers in the legend show the area under the curve (AUC). Normal exercise group responded to a lower AUC than that of others.

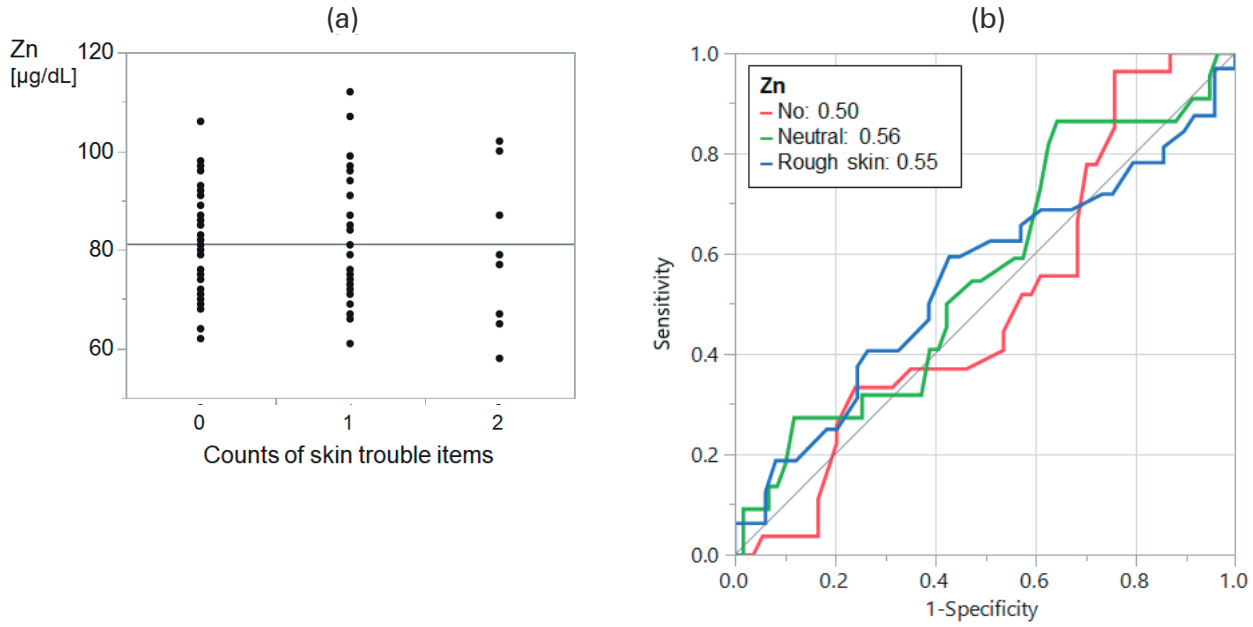

Fig. 4 (a) Univariate analysis of zinc concentration and skin-related symptoms. Horizontal axis shows counts of each individual's positive answer for rough skin and prolonged skin healing. No significant correlation was observed (p = 0.149). The horizontal line shows the average of the whole data (81.2 μg/dL).
(b) ROC curve of zinc concentration and rough skin. The numbers in the legend show AUC. No statistical tendency was observed.

The results for daily habits are shown in Fig. 2. Questions related to zinc or iron deficiency were selected as described in the literature [1,3]. The items "Feel vitality" and "Feel anemia" specifically addressed iron deficiency, whereas other questions focused on targeting zinc deficiency. The distribution of responses varied across questions, with no significant differences observed between survey results regarding habits or exercise.

The survey responses regarding serum iron and zinc deficiencies are presented in Table 4. For iron deficiency (Table 4a), no statistically significant differences were found in vitality (p = 0.2450) or anemia (p = 0.0995). Similarly, no significant differences were observed between healthy students and those with asymptomatic zinc deficiency. However, one student had symptomatic zinc deficiency (serum zinc concentration was 58 μg/dL) and reported having rough skin and prolonged healing of the skin injury.

The correlation between serum iron concentration and exercise strength is shown in Fig. 3a. A significant difference was observed between groups without exercise and those engaging in extreme exercise. Serum iron concentration in the non-exercise group was lower than in the extreme-exercise group. In contrast, no correlation was observed between the types of sports activities (data not shown). In the non-exercise group, 14 (43.8%) students answered that they did not engage in activity, 4 (12.5%) did yoga, and 3 (9.4%) had walking habits. Eighteen students (90.0 %) in the extreme exercise group participated in volleyball.

The ROC curve used to determine the iron deficiency threshold is shown in Fig. 3b. No statistical threshold was obtained for exercise habits based on the curve (AUC). However, the AUC of the non- and extreme-exercise groups were larger than those of the normal group.

The correlation between serum zinc concentration and subjective skin-related symptoms is depicted in Fig. 4. Although not statistically significant (p = 0.1489), the serum zinc concentration in participants with multiple positive skin symptoms was lower than that in participants with fewer skin symptoms (Fig. 4a). The ROC curves for serum zinc concentration and subjective skin healing did not correlate (Fig. 4b). This indicates that there is no additional information regarding the serum zinc concentration threshold for zinc deficiency.

The results of blood examinations over two consecutive years were evaluated. However, no significant differences

were observed between the groups (data not shown).

Discussion

This study demonstrates that clinical iron or zinc deficiency may influence adolescent students' perceptions. Additionally, asymptomatic zinc deficiency yielded various outcomes from the students' subjective perspectives.

No correlation was observed between the questionnaire and blood examination data, except for exercise strength and iron concentration (Fig. 3a). The results revealed a significant difference in serum iron concentrations across the exercise intensities. In contrast, no significant difference was observed in serum hemoglobin levels. Serum iron concentrations exhibit significant variations throughout the day, with Statland et al. reporting a variation of up to 12.9% [16]. The SD of serum iron concentrations in each group were 33.6 μg/dL and 42.1 μg/dL for the non-exercise and extreme exercise groups, respectively. The mean values for the non-exercise and extreme exercise groups were 87.3 μg/dL and 109.5 μg/dL, respectively. These statistical values were aligned with reported values [17].

Sandström et al. reported that the prevalence of iron deficiency among female adolescents in the athlete group was lower than that in the nonathlete group [18]. This disparity may be attributed to good iron intake and low menstrual bleeding. Another mechanism of anemia involves blood loss during menstruation or blood dilution due to the excessive intake of oral solutions [17]. Unfortunately, this information was not obtained in the present study.

The prevalence of zinc deficiency was higher than that of iron deficiency. Atasoy et al. reported zinc and iron deficiency rates of 10.9% and 6.0%, respectively [20]. The prevalence of zinc deficiency ranges (7% to 37%) [20-24], depending on the cutoff value of serum zinc concentration (50–75 μg/dL) and the cohort population. In this study, the prevalence of symptomatic zinc deficiency was 1.2%, lower than that reported in previous studies. This difference may be due to population and cutoff differences.

Regarding exercise activities (Table 3), the strength of sports activities appeared to depend on the responses to sports items. Volleyball is commonly used as an extreme exercise that reflects club participation. Because these responses were subjective, they did not correlate with laboratory data or physical examination results. The proportions in each analysis did not show any specific trends.

The results of the survey, shown in Fig. 2, included positive (good appetite and vitality), negative (rough skin, chronic skin injury, frequent infection, and anemia), and neutral (growth spurt and taste) items. Regarding positive questions, many students answered, "agree."

In this cohort, one student exhibited symptomatic zinc deficiency, tested positive for rough skin, and showed prolonged healing. Although the number of subjects was too small for statistical evaluation, symptomatic zinc deficiency could have influenced physical symptoms. No statistically significant correlation was observed between asymptomatic zinc deficiency and students' perceptions. Zinc and its related zinc-finger regulate apoptosis, folding, and assembly of proteins, and lipid binding, thereby stabilizing skin homeostasis [25]. Considering these physiological mechanisms, zinc deficiency is suspected to have affected the participants' perceptions. In addition, other factors may affect serum zinc concentrations, such as a decrease in zinc absorption in the small intestine caused by the concurrent consumption of certain types of food. Calcium and milk are examples of such foods [26]. Physical or mental stress also affects serum zinc concentration [9,27]. Therefore, we attempted to determine the cutoff values for iron and zinc levels to detect subjective symptoms (Figs. 3 and 4). The AUC for these elements was low, potentially due to the high tolerance of the blood concentration [9].

The questionnaire used in this study did not inquire about oral supplementation habits. Therefore, we could not assess the correlation between zinc deficiency and oral intake. Several studies have reported that insufficient oral zinc intake can lead to various disorders. In a randomized controlled trial, Mossad et al. reported that oral zinc intake reduced the duration of common cold symptoms [28]. Kobayashi et al. reported that 12%–13% of junior high and high school students consumed oral mineral supplements. Moreover, 32.3% of patients with eczema consumed vitamin or mineral supplements, a higher percentage than that in the healthy group in their study [29].

This study has several limitations. First, this study may have had a selection bias because the study population comprised only female students, potentially affecting iron concentration through menstrual discharge, and

the students were from a single institute. Second, participants were not asked about their oral supplement intake. Even if the sample size was sufficient, oral intake may have affected the results.

In conclusion, serum zinc concentrations may affect female adolescent students' perceptions of skin disorders. Higher serum zinc concentrations may improve the quality of life in female students by preventing skin disorders. Serum iron concentrations were higher in the extreme exercise group than in the non-exercise group. Therefore, iron intake should be approached appropriately and cautiously to prevent iron deficiency.

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Author contributions

N.Y. designed the study, collected and analyzed the data, and wrote the manuscript. C.K. performed clinical evaluations and reviewed the manuscript. S. N., Y. O., and T. S. supervised the study and revised the manuscript. All the authors have read and approved the final version of the manuscript.

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Supplementary Materials

Supplemental document: an inquiry form.

Tell us about your exercise habits.

Q1: Do you exercise on a regular basis?
☐Intensely ☐I do ☐No

Q2: How often do you exercise?
☐Almost every day ☐4–5 days a week
☐2–3 days a week ☐1 day a week
☐1–3 days a month ☐Less than that

Q3: What kind of exercise do you do? (multiple choice)
☐Volleyball
☐Dance
☐Basketball
☐Futsal/soccer
☐Badminton
☐Tennis
☐Figure skating
☐Swimming
☐Yoga/stretching
☐Walking/strolling
☐Jogging
☐Skiing/snowboarding
☐Others
☐No exercise

Q4: (Free text for "Others" in Q3)

Tell us about your physical constitution and eating habits.

Q5: Do you like salty foods?
☐I like too spicy food.
☐I like spicy food
☐I do not like spicy food
☐Neutral

Q6: Do you catch a cold easily?
☐Easily catch a cold.
☐I am not prone to catching colds.
☐Neutral

Q7: Do you have rough skin?
☐Yes
☐No
☐Neutral

Q8: Do you have difficulty in healing wounds?
☐Yes
☐No
☐Neutral

Q9: I did not grow taller during my growth period (until around age 11 for females).
☐Yes
☐No
☐Neutral

Q10: I lose my hair.
☐Yes
☐No
☐Neutral

Q11: I have no appetite.
☐Not much.
☐I have an appetite.
☐Neutral

Q12: Do you get mouth ulcers?
☐Yes
☐No
☐Neutral

Q13: I am anemic.
☐Yes
☐No
☐Neutral

Q14: I always feel energy.
☐Yes
☐No
☐Neutral