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Association of Underweight to Low Lymphocyte Count and Prealbumin as Indicators of Malnutrition in Japanese Women

（女性の痩せと栄養指標であるリンパ球数およびプレアルブミンとの関連）

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Abstract

Objective

Although underweight women potentially have their health problems, the number of underweight among young women has increased in Japan. The aim of the present study was to investigate the nutritional problems of young Japanese women by measuring serum albumin, prealbumin and lymphocyte count as indicators of nutritional status.

Methods

Study 1 (the pilot study): The subjects were 114 women aged 20-39 who participated in an annual health check-up for residents in K city of Aichi, Japan. Data from questionnaire, physical measurements and blood tests were analyzed in relation to severe underweight (BMI $\leq$17.5 kg/m^2), slight underweight (17.5< BMI <18.5 kg/m^2), normal weight (18.5$\leq$ BMI <25 kg/m^2), and obesity (BMI $\geq$25 kg/m^2).

Study 2 (the main study): The subjects were 912 women aged 19-39 years who participated in an annual health check-up for residents in N city of Aichi, Japan.

Questionnaire data, physical measurements and blood tests were analyzed in relation to weight categories of underweight (body mass index (BMI) <18.5 kg/m^2), normal (18.5≤ BMI <25 kg/m^2), and obese (BMI ≥25 kg/m^2).

Results

Study 1 (the pilot study): Lymphocyte count tended to be lower with a decrease in BMI. The prevalence of low lymphocyte count (<1500 /µl) increased with underweight. In subjects who had restricted food intake for weight loss, leukocyte count and serum total protein as well as lymphocyte count were lower. A multivariate logistic regression analysis showed the association of low lymphocyte count to severe underweight (odds ratio (OR): 1.95; 95% confidence interval (CI): 1.07-3.56) and restricted food intake for weight loss (OR: 3.73; 95% CI: 0.91-15.30).

Study 2 (the main study): Lymphocyte counts were significantly lower in the underweight group. A multivariate logistic regression analysis showed an association of low lymphocyte count (<1500 /µl) with underweight (OR 1.96, 95% CI 1.35−2.83). Low prealbumin (<20 mg/dl) was associated with weight loss (OR 1.42, 95% CI 1.00−2.02), but not with underweight. Albumin was not lower in the underweight group. The prevalence of low lymphocyte count was higher by 35% among underweight women who lost weight of 1 kg or more in the past three months, and higher by 50% among those who lost weight of over 2 kg.

Conclusions

The present study suggests that underweight women in their 20s and 30s can be at risk for low lymphocyte count or potential malnutrition. The risk is thought to be high, particularly in underweight women who additionally lose weight in recent months.

Introduction

The desire to be thin is widespread among young women in Japan. Japanese women are likely to have a desire to be thin even when they have a lower BMI than women in other countries.[1,2] The number of underweight women has recently increased, and more than 20% of Japanese women in their 20s are underweight.[3] The Japanese government has adopted a 10-year national plan called 'Healthy Japan 21' in which one of the objectives is to reduce the prevalence of underweight (BMI <18.5 kg/m^2) in women in their 20s to less than 15%. Thus, being underweight is a health problem among young women in Japan.

Among health problems of underweight women, anorexia nervosa has been studied from various points of view, such as immunity function,[4] energy metabolism,[5] and liver function.[6] Patients with anorexia nervosa also tend to have amenorrhea[7] and low bone mass.[8] However, there are few studies on underweight women who do *not* suffer from anorexia nervosa.

Recent studies have shown that being underweight at pre-pregnancy is not only a health problem for women themselves but also a risk factor affecting their infants. A low body mass index (BMI) among pre-pregnancy women is a risk factor for fetal growth deficits,[9,10] preterm birth[11] and spontaneous abortion.[12] A previous study reported that the risks of low birth-weight infants and hospitalization of the infants rose as women had a pre-pregnancy weight of BMI <18.5 kg/m^2, though these risks were not associated with being underweight during pregnancy.[13] Thus, underweight pre-pregnant women in their 20s to 30s can be at risk for both their health and growth of the fetus.

Underweight women in their 20s and 30s appear healthy, but may have potential health problems. The present study conducted to demonstrate nutritional problems among underweight women.

As for the relationship between underweight and the leukocyte count, previous studies have reported a decline in the leukocyte and lymphocyte count in patients with anorexia nervosa, who were severely thin.[14,15] The lymphocyte count is known to decrease in a poor nutritional state, due especially to protein-energy malnutrition, and it is regarded as a nutritional state indicator.[16] Malnutrition may be one of the health problems for underweight women. However, there are few studies on lymphocyte count among underweight women, who have not suffered from anorexia nervosa. The aim of the first study (*Study 1; the pilot study*) was to clear the relationship between underweight and lymphocyte count as an indicator of the nutritional status in young Japanese women.

Albumin, prealbumin and lymphocyte count are regarded as indicators of malnutrition,[16-18] and are used as popular markers of nutrition screening for hospital inpatients.[19-22] However, there are few studies on albumin, prealbumin and lymphocyte count among underweight women. The aim of the second study (*Study 2; the main study*) was to investigate the health problems of Japanese underweight women in their 20s and 30s who underwent a health check-up by measuring albumin, prealbumin and lymphocyte count as indicators of their nutritional status.

Study 1; the pilot study

Relationship between underweight and the lymphocyte count

Methods and Results

Methods (*Study 1*)

A cross-sectional study was conducted on population-based samples. The subjects were female participants aged 20-39 in an annual health check-up for residents conducted November 2 to 9, 2005 in K city of Aichi, Japan. A total of 195 women underwent the health check-up. Among them, 180 persons (response rate: 92.3%) consented to participate in this study. This study has been approved by the ethics committee of the Nagoya University School of Medicine.

The examinations including blood tests were all conducted in the morning from 9 to 12 o'clock. BMI (kg/m^2) was calculated as weight in kilograms divided by the square of the height in meters. Blood samples were measured at the laboratory in the Handa Medical Health Care Center (Handa City, Aichi, Japan). Blood counts of leukocytes and lymphocytes were done by automatic cell counter (Sysmex SE-9000). Differential leukocyte count was obtained by microscopic counts. Serum concentrations of total protein were measured by a clinical autoanalyzer (HITACHI 7700). A self-reported questionnaire was conducted on clinical history and lifestyle included smoking, alcohol drinking habit, and restricted food intake for weight loss.

Smoking habit was asked by “Never,” “Former” or “Current.” Alcohol drinking was asked on the basis of frequency: “4 days or more a week,” “1 to 3 days a week,” “Occasionally” or “None.” Menstrual cycle was questioned by “Regular,” “Sometimes irregular,” “Irregular” or “Amenorrhea.” Restricted food intake for weight loss was asked by “not restricting food intake,” “wish to restrict, but not actually” or “be restricting.” With respect to restricted food intake, subjects were categorized into the food-intake restricting group and the non-restricting group.

The subjects with amenorrhea because of the possibility of pregnancy, current smokers, alcohol drinkers of one day or more a week and persons with a cold or arthritis rheumatism were excluded from the present analysis because leukocyte count is affected.[23-26] Another one subject having a leukocyte count of 13600 /μl was excluded because of possible infection or inflammation. There were no persons having lifestyle related diseases such as diabetes and hyperlipemia.

As a result, there remained 114 participants (mean ± standard deviation (SD): 33.3 ± 3.8 years) for the present analysis. Their BMIs were 20.1 ± 2.2 kg/m^2 in their 20s and 20.7 ± 3.0 kg/m^2 in their 30s, which did not differ much from those of the National Nutrition Survey 2003 in Japan (20.7 ± 3.1 kg/m^2 and 21.4 ± 3.6 kg/m^2, respectively).

According to the criteria of the Japan Society for the Study of Obesity, BMI was categorized into underweight (BMI <18.5 kg/m^2), normal weight (18.5≤ BMI <25 kg/m^2) and obesity (BMI ≥25 kg/m^2). In addition, in the International Statistical Classification of Diseases and Related Health Problems Tenth Revision (ICD-10), BMI of 17.5 kg/m^2 or less is one of the criteria for anorexia nervosa.[27] Therefore, in the present study, underweight was further classified into slightly underweight (17.5< BMI <18.5 kg/m^2) and severely underweight (BMI ≤17.5 kg/m^2). A low lymphocyte count of <1500 count/μl was defined as low, based on nutritional assessment criteria.[16]

A statistical analysis was made as follows. The linear trend, Student's *t*-test, Fisher's exact probability test and Chi-square for trend were used to analyze the statistical differences in physical data among the BMI groups and between the food-intake restricting group and the non-restricting group. The association of low lymphocyte count with underweight and restricted food intake was investigated with a logistic regression analysis after adjusting for age. The p values of < 0.05 were considered statistically significant, and the p values from 0.05 to 0.10 were regarded borderline significant. All statistical analyses were completed with the statistical package SPSS 14.0 J for Windows.

Results (*Study 1*)

As shown in Table 1, the subjects were classified into 10 (8.8%) severely underweight, 13 (11.4%) slightly underweight, 81 (71.1%) normal weight, and 10 (8.8%) with obesity. The prevalence of underweight (BMI < 18.5 kg/m^2) was 20.2% in total. There was no difference in age among the BMI groups. Among blood examinations, the lymphocyte count tended to be lower with a decrease in BMI (trend p = 0.058). Leukocyte count, neutrophil count and serum total protein did not differ among the BMI groups. Comparing the two groups according to restricted food intake, BMI was higher in the restricting subjects (p = 0.050), as obese subjects were more likely to restrict food intake for weight loss (Table 2). Serum total protein, leukocyte count and lymphocyte count were significantly lower in the restricting subjects ($p < 0.05$).

The prevalence of low lymphocyte count of less than 1500 /μl increased with underweight (trend p =0.049); the prevalence was 40 % in the severe underweight group (Table 3). A multivariate logistic regression analysis showed an association of low lymphocyte count to severely underweight (odds ratio (OR): 1.95; 95% confidence interval (CI): 1.07 - 3.56) and restricted food intake for weight loss (OR:

3.73; 95% CI: 0.91 - 15.30).

In 7 normal-weight persons restricting food intake, the lymphocyte count showed a low value of 1477.9 ± 221.1 /μl, and a low lymphocyte count of < 1500 /μl was found in 3 (42.9%) of them. One underweight subject who had restricted food intake had a very low lymphocyte count of 928 /μl.

Study 2; the main study

Relationship between underweight and albumin, prealbumin, lymphocyte count

Methods and Results

Methods (*Study 2*)

Design and sample

A cross-sectional study was conducted with population-based samples. The subjects were recruited from females aged 18-39 years who participated in an annual health check-up from November 6 to 27, 2008, for residents of N city in Aichi prefecture, Japan. A total of 1295 women aged 18-39 years underwent the health check-up. Among them, 1184 consented to participate in this study (response rate: 91.4%). This study was approved by the ethics committee of the Nagoya University School of Medicine.

Albumin, prealbumin and lymphocyte counts are affected by various diseases such as inflammation, infection, liver disease, renal failure, thyroid disease and others.[16,17] One hundred fifteen subjects under medical treatment were excluded from the present analysis (e.g., 20 patients with allergic dermatitis, 19 gynecoloycal diseases, 10 psychiatric diseases, 10 anemia, 9 cold, 6 sinusitis, 4 asthma, 3 hyperthyrea). Moreover, 21 pregnant women and 135 subjects currently breast-feeding were also excluded, because pregnancy and breastfeeding may affect serum protein level and leukocyte count.[23,28]

One subject with a leukocyte count of 18100/μl was excluded because of possible infection or inflammation. This left 912 participants (mean age (SD): 33.6 (4.0) years, range 19-39 years) eligible for the present analysis. Their mean of BMIs (SD) were: 20.1 (3.0) kg/m^2 in their 20s (including one subject aged 19); and 20.7 (3.1) kg/m^2 in their 30s, which did not differ much from those of the National Nutrition Survey 2005 in Japan (20.5 (2.7) kg/m^2 and 21.4 (3.8) kg/m^2, respectively).

Measurements

The health check-up included a questionnaire about clinical history and lifestyle, body height and weight measurements, and blood examinations. BMI (kg/m^2) was calculated as weight in kilograms divided by the square of height in meters.

Blood samples were obtained from the antecubital vein of a seated subject, and on the same day they were analyzed at the laboratory of the Handa Medical Health Care Center (Handa City, Aichi, Japan). Lymphocyte counts and other differential leukocyte counts were measured by an automatic cell counter (ADVIA 120, Bayer). Serum concentrations of total protein and albumin were measured by a clinical autoanalyzer (HITACHI 7700), using the Bromcresol Green method. Serum prealbumin was measured with a nephelometric assay (Behring Nephelometer II, Siemens Healthcare Diagnostics)

The self-administered questionnaire included questions on smoking, drinking habits, pregnancy, breast-feeding and weight change. The questionnaires were mainly completed at home by the participants and then checked by nurses to fill in any missing items. Smoking responses were denoted as "Never," "Former," or "Current." Alcohol consumption was classified on the basis of frequency of drinking: "4 d/week or more," "1 to 3 d/week," "Occasionally," or "None." Responses to pregnancy and breast-feeding were "Yes," or "No." The self-assessment of body shape included responses of "Thin," "Just good," or "Overweight." Desire for weight change was indicated by: "Want to lose weight," "Remain the same," or "Want to gain weight." Dieting to lose weight was indicated by: "Dieting now," "Have dieted," or "Never dieted." The question: "Has your weight changed in the last 3 months?" was responded to with "Weight loss," "No change," or "Weight gain." If they responded "weight loss," or "weight gain," the amount of the weight change was also asked. Weight change of over 1 kg was regarded as weight loss or gain.

Data analysis

According to the criteria of the Japan Society for the Study of Obesity and WHO,[29] BMI was categorized as underweight (BMI <18.5 kg/m^2), normal weight ($18.5 \leq$ BMI <25 kg/m^2) and obese (BMI ≥ 25 kg/m^2). Weight change in the last 3 months was categorized as weight loss (of 1 kg and over), no change (within 1 kg) and weight gain (of 1 kg and over). Nutritional assessments were done on the basis of the cut-off values: albumin of <3.5 g/dl, prealbumin of <20 mg/dl, and a lymphocyte count of <1500 /µl. Albumin of <3.5 g/dl and a lymphocyte count of <1500 /µl are popularly regarded as criteria during nutritional screening of hospital inpatients.[20,22,30] Though there is no common nutritional screening criterion for prealbumin,[16,21,31] we adopted a prealbumin level of <20 mg/dl according to a previous study that investigated the effectiveness of nutrition screening by prealbumin for hospitalized patients.[19]

Statistical analyses were performed as follows. The linear trend and chi-square for trend were used to analyze the statistical differences in physical data and lifestyles among both the BMI and weight change groups. Additionally, continuous measurement values were compared with the underweight or obese groups with reference to the normal weight group by Dunnett's multiple comparison test. Multivariate logistic regression analysis was also conducted in association low nutrition indicators with underweight and/or weight loss after adjusting for age and other related factors. p values of < 0.05 were considered statistically significant. All statistical analyses were completed with the statistical package SPSS 14.0J for Windows.

Results (*Study 2*)

As shown in Table 4, the prevalence of underweight (BMI <18.5 kg/m^2) was 23.0% (*n* = 210); normal weight (18.5≤ BMI <25 kg/m^2) was 68.3% (*n* = 623); and obese (BMI ≥ 25 kg/m^2) was 8.7% (*n* = 79). The age was lower in the lower BMI group ($p = 0.002$). As for blood examinations, the leukocyte ($p < 0.001$), neutrophil ($p = 0.001$), monocyte ($p = 0.004$), and lymphocyte counts ($p < 0.001$) declined with a decrease in BMI. Mean lymphocyte counts were lower in the underweight group and higher in the obese group compared with the normal weight group ($p = 0.003$, and $p < 0.001$, respectively). However, leukocyte, neutrophil and monocyte counts did not differ between the underweight and the normal weight group, though they were different between the obese and the normal weight group ($p < 0.001$). Serum prealbumin also decreased with lower BMI ($p < 0.001$). The prevalence of low lymphocyte counts of less than 1500 /μl was higher in the underweight group ($p = 0.001$). The prevalence of a low prealbumin level of less than 20 mg/dl tended to be greater among the underweight group, though not significantly ($p = 0.098$). Serum albumin increased with lower BMI ($p = 0.009$), but no one displayed a low albumin level of less than 3.5

g/dl. Serum total protein did not differ among the BMI groups.

There were 44 (21.0%) persons who wanted to lose weight in the underweight group (Table 5). Eighty-four (40.0%) had tried to diet before and 6 (2.9%) were currently dieting in the underweight group. Among 31 underweight women who lost weight of 1 kg or more in the last 3 months, 8 (25.8%) persons wanted to lose more weight, and 11 (35.5%) had tried to diet before.

In a comparison among the weight change groups (Table 6), BMI tended to be lower in the weight loss group ($p < 0.001$). Weight loss was encountered in 31 subjects (14.8%) in the underweight group. The leukocyte ($p < 0.001$), neutrophil ($p = 0.007$), monocyte count ($p = 0.004$), and lymphocyte counts ($p < 0.001$) were lower in weight-loss subjects. Serum prealbumin was lower in the weight loss group ($p < 0.001$), while serum albumin was higher in the weight loss group ($p = 0.012$). Serum total protein did not differ among the weight change groups. The prevalence of low prealbumin of less than 20 mg/dl increased with weight loss ($p = 0.038$), while that of low lymphocyte counts of less than 1500 /μl did not differ among the weight change groups.

Smoking habit and alcohol consumption did not differ among the BMI groups and weight change groups. However, a smoking habit affected total protein ($p < 0.001$), prealbumin ($p < 0.001$), leukocyte counts ($p < 0.001$) and lymphocyte counts ($p < 0.001$) (data not shown). Alcohol consumption was also associated with total protein ($p = 0.003$), prealbumin ($p < 0.001$) and leukocyte counts ($p = 0.039$) (data not shown).

The possible association of being underweight and weight loss with a low lymphocyte count of less than 1500/μl or low prealbumin of less than 20 mg/dl was examined by multivariate logistic regression analysis after adjusting for age, smoking and drinking habits (Table 7). The multivariate logistic regression analysis showed an association of low lymphocyte counts (< 1500/μl) with being underweight (odds ratio (OR) 1.96, 95% confidence interval (CI) 1.35−2.83), but not with weight loss in the last 3 months. On the other hand, low prealbumin (<20 mg/dl) was associated with weight loss (OR 1.42, 95%CI 1.00−2.02), but not with being underweight.

In relation to the BMI and weight loss groups, the prevalence of low lymphocyte counts and that of low prealbumin levels were examined among 647 subjects who had neither smoking nor drinking habits to exclude the influence of the two habits (Table 8). Low lymphocyte counts (<1500 /μl) were most frequently found in 7 (35%) in the underweight plus weight loss groups ($n = 20$). Further, 4 (50.0%) had low lymphocyte counts (<1500 /μl) among 8 underweight women who lost weight of 2 kg or more in the last 3 months. The prevalence of low lymphocyte counts tended to be higher with lower BMI in the weight loss subjects (p = 0.077) or those without weight change (p = 0.039). On the other hand, the prevalence of low prealbumin (<20 mg/dl) was not at its highest in the underweight plus weight loss groups (Table 9). The prevalence tended to be higher in the weight loss group in the normal weight (p = 0.010) and obese subjects (p = 0.023).

Discussion

Study 1 and *Study 2*

Discussion

Serum concentrations of albumin, prealbumin and lymphocyte counts are often used as an indicator of nutritional status. The present studies examined those three indicators among young adult Japanese woman participants of an annual health checkup, to investigate their nutritional status, particularly those of underweight women. The prevalence of being underweight (BMI <18.5 kg/m^2) was 20.2% in the *Study 1* and 23.0% in the *Study 2* population in their 20s and 30s, which was similar to the 22.6% in their 20s and 20.0% in their 30s in the National Nutrition Survey 2005. The present studies showed that underweight women were likely to have lower lymphocyte counts. The prevalence of a low lymphocyte count (<1500 /μl) tended to be higher in the underweight subjects.

Especially in severely underweight women in the *Study 1*, the low lymphocyte count was encountered in 40 % of them. The low lymphocyte count was also found in 43% of the food-intake restricting people with normal weight. In the *Study 2*, the prevalence was higher by 35% among underweight women who lost weight of 1 kg or more in the past three months, and higher by 50% among those who lost weight of over 2 kg. These findings suggested that underweight women were at risk for low lymphocyte counts.

Protein-energy malnutrition (PEM) is known to decrease lymphocyte counts,[32-34] and lymphocyte counts are used as an indicator of PEM.[16] Severely underweight patients with anorexia nervosa reportedly have lower lymphocyte counts.[4,15,35] The present studies demonstrated that underweight women had a higher risk of a lower lymphocyte count, and that only the lymphocyte counts among the differential counts of leukocytes were significantly decreased in the underweight group compared with the normal weight group. Multivariate logistic regression analysis showed an association of low lymphocyte counts (<1500 /μl) with the underweight group (*Study 1*: OR 1.95, 95% CI 1.07 - 3.56, *Study 2*; OR 1.96, 95% CI 1.35−2.83). Low lymphocyte counts in underweight women aged 20-30s may reflect their potential malnutrition.

The two studies demonstrated that weight loss and restricted food intake for weight loss in underweight women might increase the risk of low lymphocyte count. In the *Study 1*, a multivariate logistic regression analysis showed the association of low lymphocyte count with restricted food intake for weight loss (OR: 3.73; 95% CI: 0.91 - 15.30) and with underweight (OR: 1.95; 95% CI: 1.07 - 3.56), showing that restricted food intake as well as underweight may carry a higher risk of a low lymphocyte count. In particular, a low lymphocyte count was found in 43% of normal or underweight persons with restricted food intake. One underweight person who had restricted food intake had an extremely low lymphocyte count of 928 /μl. Restriction of food intake for weight loss in underweight women appears to increase the risk of low lymphocyte count. In the *Study 2*, Weight loss in these months seemed to enhance the decrease of lymphocyte counts. A low lymphocyte count (<1500 /μl) was most frequently found in 35% of the underweight women who additionally had lost weight in the past three months. An increased amount of weight loss led to a higher prevalence of low lymphocyte counts. Among 8 underweight women with weight loss of 2 kg or more, 50.0% had low lymphocyte counts. These findings suggested that weight loss in underweight women may increase the risk of malnutrition. The risk of malnutrition is thought to be high, particularly in underweight women who additionally lose weight in recent months.

In our present study (*Study 2*), albumin and prealbumin showed no clear association with being underweight. Concentrations of serum albumin were not lower in the underweight group, and no one displayed a low albumin level of less than 3.5 g/dl. Serum total protein did not differ among the BMI groups. Serum prealbumin was lower in the underweight group, but the present multivariate logistic regression analysis did not show any association between a low prealbumin level of <20 mg/dl and being underweight. It may be difficult to assess malnutrition by albumin and prealbumin. Earlier studies have reported that concentrations of albumin and prealbumin were within the normal range even in patients with anorexia nervosa.[14,17,36] Other studies have also demonstrated that albumin and prealbumin concentrations did not differ between patients with anorexia nervosa and the controls.[37,38] Thus, albumin and prealbumin levels may not be useful in assessing the nutritional status of underweight and anorexia nervosa patients. Some sort of compensatory mechanisms might work to preserve serum concentrations of albumin and prealbumin in the case of those with anorexia nervosa or severely underweight subjects. The present study suggested that lymphocyte counts could serve as a more sensitive indicator of nutritional status in underweight women than either serum prealbumin or albumin.

A multivariate logistic regression analysis showed a closer association of low prealbumin (<20 mg/dl) with weight loss, rather than with being underweight. The prevalence of low prealbumin was not at its highest in the underweight plus weight loss groups, though the prevalence tended to be higher in the weight loss group. Prealbumin has a shorter half-life of approximately 2.5 d and reflects the changes in protein nutritional status.[17] A previous study has reported that the concentration of prealbumin increased after a weight increase in those with anorexia nervosa.[36] These findings may explain why prealbumin was more closely associated with weight loss in these months. Meanwhile, lymphocyte counts showed a close relationship with being underweight, but not with weight loss. However, weight loss in underweight women increased the risk of low lymphocyte counts. A low lymphocyte count (<1500 /μl) was most frequently found in 35% of underweight women who had lost additional weight in the last three months, while it was encountered in around 17% of underweight women without weight loss.

In Japan, the desire to be thin is widespread among young women. The present result also showed that 21% of underweight women wanted to lose more weight, and 43% were currently dieting or had in the past (*Study 2*). Thus, even underweight women had the desire to be thin and to diet. As mentioned above, weight loss and restricted food intake for weight loss in underweight women leads to an increased risk of malnutrition. Potential malnutrition among pre-pregnancy women may cause fetal health problems[9-11,13] as well as their own health problems. It is important that especially underweight women should not try to lose weight by needless dieting, to prevent risk of malnutrition.

Lymphocyte counts may indicate not only a subject's nutritional state but also her immune function. It is well documented that protein-energy malnutrition can also cause serious immunological deficits.[34] Thus, it is hypothesized that underweight women with low lymphocyte counts may suffer from some impaired immune function. However, the results of earlier studies did not clearly demonstrate immunological deficiencies in cases of anorexia nervosa.[4,15,39,40] The present study showed low lymphocyte counts in underweight women who lost weight recently. However, our studies did not measure lymphocyte subsets. Further studies will be required on the immune function in underweight women.

The present study had some limitations. Our subjects were limited to participants in a health check-up in a single city in central Japan. Since weight change was asked via a self-reported questionnaire, we may not have reliably elicited exact answers on that subject. In addition, there was rather a small number of underweight persons who had lost weight recently. Despite these limitations the present study suggested that the risk of malnutrition is likely to be high in women who were underweight and additionally lost weight in recent months. Further studies are warranted to obtain more conclusive results.

Conclusions

The present study showed that underweight women were likely to have lower lymphocyte counts. The prevalence of a low lymphocyte count (<1500 /μl) tended to be higher in the underweight subjects. The prevalence was higher by 35% among underweight women who lost weight of 1 kg or more in the past three months, and higher by 50% among those who lost weight of over 2 kg. These findings suggested that underweight women in their 20s and 30s can be at risk for low lymphocyte count or potential malnutrition. The risk for malnutrition is thought to be particularly high in underweight women who have lost weight recently.

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Table 1. Characteristics according to BMI groups

	Severely underweight (*n* =10)	*Slightly underweight* (*n* =13)	*Normal* (*n* =81)	*Obese* (*n* =10)	trend *p**
Total protein (g/dl)	7.67 ± 0.16	7.56 ± 0.28	7.48 ± 0.35	7.52 ± 0.33	0.144
Leukocyte ($\times 10^3/\mu l$)	5.71 ± 1.48	5.69 ± 1.39	5.72 ± 1.36	6.44 ± 1.29	0.384
Neutrophil ($\times 10^3/\mu l$)	3.63 ± 1.09	3.46 ± 0.95	3.40 ± 0.92	3.73 ± 0.67	0.904
Lymphocyte ($\times 10^3/\mu l$)	1.68 ± 0.54	1.75 ± 0.43	1.85 ± 0.46	2.07 ± 0.34	0.058
BMI (kg/m^2)	16.6 ± 0.63	18.0 ± 0.29	20.7 ± 1.55	27.2 ± 2.57	−

Severely underweight (BMI≤17.5 kg/m^2), Slightly underweight (17.5<BMI<18.5 kg/m^2), Normal (18.5≤BMI＜25 kg/m^2), Obese (BMI≥25 kg/m^2).

Data are expressed as mean ± SD.

* The trend *p* values by the linear trend.

Table 2. Characteristics according to Restricted food intake

	Restricting (n =10)	*Not restricting* (n =104)	p*
Total protein (g/dl)	7.21 ± 0.38	7.54 ± 0.32	0.002
Leukocyte ($\times 10^3/\mu l$)	4.95 ± 1.17	5.86 ± 1.36	0.044
Neutrophil ($\times 10^3/\mu l$)	2.95 ± 0.68	3.50 ± 0.92	0.066
Lymphocyte ($\times 10^3/\mu l$)	1.54 ± 0.38	1.87 ± 0.46	0.030
BMI (kg/m^2)	22.3 ± 4.84	20.5 ± 2.61	0.050

Data are expressed as mean ± SD.

* The p values by Student's t-test.

Table 3. Association of low lymphocyte count (< 1500 /µl) with underweight and restricted food intake

	Lymphocyte count				
	Normal (≥1500 /µl)	*Low* (<1500 /µl)	*p**	*Multivariate OR*** *(95% CI)*	*p*
BMI groups					
Severely underweight	6 (60.0)	4 (40.0)	0.049	1.95 (1.07 - 3.56)	0.030
Slightly underweight	10 (76.9)	3 (23.1)			
Normal	64 (79.0)	17 (21.0)			
Obese	10 (100.0)	0 (0.0)			
Restricted food intake					
Restricting	6 (60.0)	4 (40.0)	0.213	3.73 (0.91- 15.30)	0.067
Not restricting	84 (80.8)	20 (19.2)			

Data are expressed as frequency (%), Odds ratio (OR) and 95% confidential interval (CI) of logistic regression analysis.

Severely underweight (BMI≤17.5 kg/m^2), Slightly underweight (17.5<BMI<18.5 kg/m^2), Normal (18.5≤BMI＜25 kg/m^2), Obese (BMI≥25 kg/m^2).

* The *p* values by Chi-square for trend or Fisher's exact probability test .

** Multivariate OR was adjusted for age, restricted food intake and the classified BMI.

Table 4. Examination results according to BMI groups

	Underweight (n = 210)		*Normal* (n = 623)		*Obese* (n = 79)		
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	p^{a}
Age (year)	29.9	5.31	31.1	4.94	31.6	4.77	0.002
Total protein (g/dl)	7.37	0.35	7.37	0.36	7.37	0.35	0.989
Albumin (g/dl)	4.49	0.20	4.45	0.21	4.44	0.21	0.009
Prealbumin (mg/dl)	23.3	3.56	23.8	3.64	25.8	3.95	< 0.001
Leukocyte count (/µl)	6250.5	1607.9	6473.5	162. 6	7574.7	1886.8	< 0.001
Neutrophil count (/µl)	3771.5	1368.2	3873.8	1335.8	4511.0	1632.6	0.001
Lymphocyte count (/µl)	1904.0	514.8	2041.0	533.8	2419.8	628.6	< 0.001
BMI (kg/m^2)	17.5	0.78	20.8	1.61	27.9	2.63	
	n	*%*	*n*	*%*	*n*	*%*	p^{a}
Prealbumin							
Low (<20 mg/dl)	33	15.7	91	14.6	5	6.3	0.098
Normal (≥20 mg/dl)	177	84.3	532	85.4	74	93.7	
Lymphocyte count							
Low (<1500 /µl)	38	18.1	79	12.7	2	2.5	0.001
Normal (≥1500 /µl)	172	81.9	544	87.3	77	97.5	
Weight change in last 3 months							
Weight loss	31	14.8	76	12.2	11	13.9	0.002
No change	163	77.6	411	66.0	51	64.6	
Weight gain	16	7.6	136	21.8	17	21.5	

Underweight (BMI <18.5 kg/m^2), Normal (18.5≤ BMI <25 kg/m^2), Obese (BMI ≥25 kg/m^2).

Data are expressed as mean (SD) and frequency (%).

[a] *p* values by linear trend and chi-square for trend.

Table 5. Examination results according to BMI groups

	Underweight (n = 210)		*Normal* (n = 623)		*Obese* (n = 79)		
	n	%	*n*	%	*n*	%	p^a
Smoking							
Never	157	74.8	495	79.5	59	74.7	0.476
Former	18	8.6	59	9.5	6	7.6	
Current	35	16.7	69	11.1	14	17.7	
Alcohol consumption							
None	134	63.8	366	58.7	48	60.8	0.675
Occasionally	37	17.6	115	18.5	18	22.8	
One to three d/week	19	9.0	69	11.1	9	11.4	
Four d/week or more	20	9.5	73	11.7	4	5.1	
Perceived body weight							
Thin	60	28.6	6	1.0	0	0.0	< 0.001
Just right	137	65.2	274	44.1	0	0.0	
Overweight	13	6.2	342	55.0	79	100.0	
Desire for weight change							
Want to loss weight	44	21.0	495	79.5	78	98.7	< 0.001
Remain the same	141	67.1	126	20.2	0	0.0	
Want to gain weight	25	11.9	2	0.3	1	1.3	
Diet							
Be dieting now	6	2.9	118	19.0	23	29.1	< 0.001
Have experience of diet	84	40.0	317	51.0	45	57.0	
Never diet	120	57.1	186	30.0	11	13.9	

Underweight (BMI <18.5 kg/m^2), Normal (18.5≤ BMI <25 kg/m^2), Obese (BMI ≥25 kg/m^2).

Data are expressed as frequency (%).

[a] p values by chi-square for trend.

Table 6. Examination results according to weight change

	Weight loss (n = 118)		*No change* (n = 625)		*Weight gain* (n = 169)		
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	p^a
Age (year)	32.9	4.29	33.8	4.05	33.6	3.67	0.001
Total protein (g/dl)	7.32	0.35	7.39	0.35	7.30	0.37	0.553
Albumin (g/dl)	4.45	0.21	4.46	0.21	4.43	0.21	0.012
Prealbumin (mg/dl)	23.1	3.84	23.9	3.62	24.2	3.87	< 0.001
Leukocyte count (/µl)	6122.9	1608.4	6515.7	1629.2	6800.0	1850.4	< 0.001
Neutrophil count (/µl)	3556.7	1242.9	3934.3	1364.2	4042.3	1508.6	< 0.001
Lymphocyte count (/µl)	2030.6	520.9	2013.4	530.3	2157.1	639.6	< 0.001
BMI (kg/m^2)	20.7	3.33	20.4	2.98	21.4	3.09	< 0.001
	n	*%*	*n*	*%*	*n*	*%*	p^a
Prealbumin							
Low (<20 mg/dl)	22	18.6	90	14.4	17	10.1	0.038
Normal (≥20 mg/dl)	96	81.4	535	85.6	152	89.9	
Lymphocyte count							
Low (<1500 /µl)	16	13.6	83	13.3	20	11.8	0.640
Normal (≥1500 /µl)	102	86.4	542	86.7	149	88.2	

Weight change was clarified according to weight changes in the previous three months.

Data are expressed as mean (SD) and frequency (%).

[a] *p* values by the linear trend and chi-square for trend.

Table 7. Odds ratio (OR) and 95% confidential interval (CI) of multivariate logistic regression analysis in association of low lymphocyte count or low prealbumin with underweight and weight change

	Multivariate OR	*95% CI*	*p*
Low lymphocyte count (<1500 /μl)			
Age	1.03	0.98−1.08	0.303
BMI groups[a]	1.96	1.35−2.83	< 0.001
Weight change[b]	1.00	0.70−1.45	0.984
Smoking[c]	0.54	0.37−0.81	0.003
Alcohol consumption[d]	0.96	0.78−1.18	0.670
Low prealbumin (<20 mg/dl)			
Age	1.01	0.97−1.06	0.567
BMI groups[a]	1.30	0.92−1.83	0.142
Weight change[b]	1.42	1.00−2.02	0.049
Smoking[c]	0.83	0.61−1.15	0.264
Alcohol consumption[d]	0.69	0.55−0.87	0.002

[a] BMI groups: "1; obese (BMI ≥25 kg/m^2)," "2; normal (18.5≤ BMI <25 kg/m^2)," "3; underweight (BMI <18.5 kg/m^2)."

[b] Weight change: "1; weight gain," "2; no change," "3; weight loss."

[c] Smoking: "1; Never," "2; Former," "3; Current."

[d] Alcohol consumption: "1; None," "2; Occasionally," "3; one to three d/week," "4; four d/week or more."

Table 8. Frequency (%) of low lymphocyte count according to underweight and weight change groups

	Underweight		*Normal weight*		*Obese*		
	n	*%*	*n*	*%*	*n*	*%*	p^a
Weight loss							
Low lymphocyte	7	35.0	7	13.0	1	16.7	0.077
Normal lymphocyte	13	65.0	47	87.0	5	83.3	
No change							
Low lymphocyte	20	17.5	43	14.1	1	2.6	0.039
Normal lymphocyte	94	82.5	261	85.9	38	97.4	
Weight gain							
Low lymphocyte	2	16.7	14	16.5	0	0.0	0.226
Normal lymphocyte	10	83.3	71	83.5	13	100.0	
p^b	0.129		0.540		0.113		

647 subjects with no habits of smoking and alcohol consumption were used for analysis.

Underweight (BMI <18.5 kg/m^2), Normal (18.5≤ BMI <25 kg/m^2), Obese (BMI ≥25 kg/m^2). Weight change was classified according to weight changes in the previous three months. Low lymphocyte (<1500 /µl), Normal lymphocyte (≥1500 /µl).

[a] *p* values by chi-square for trend between indicators of malnutrition with BMI in each weight change group.

[b] *p* values by chi-square for trend between indicators of malnutrition with weight changes in each BMI group.

Table 9. Frequency (%) of low prealbumin according to underweight and weight change groups

	Underweight		*Normal weight*		*Obese*		
	n	*%*	*n*	*%*	*n*	*%*	p^a
Weight loss							
Low prealbumin	4	20.0	12	22.2	2	33.3	0.573
Normal prealbumin	16	80.0	42	77.8	4	66.7	
No change							
Low prealbumin	20	17.5	57	18.8	2	5.1	0.262
Normal prealbumin	94	82.5	247	81.3	37	94.9	
Weight gain							
Low prealbumin	2	16.7	6	7.1	0	0.0	0.112
Normal prealbumin	10	83.3	79	92.9	13	100.0	
p^b	0.790		0.010		0.023		

647 subjects with no habits of smoking and alcohol consumption were used for analysis.

Underweight (BMI <18.5 kg/m^2), Normal (18.5≤ BMI <25 kg/m^2), Obese (BMI ≥25 kg/m^2). Weight change was classified according to weight changes in the previous three months. Low prealbumin (<20 mg/dl), Normal prealbumin (≥20 mg/dl).

[a] *p* values by chi-square for trend between indicators of malnutrition with BMI in each weight change group.

[b] *p* values by chi-square for trend between indicators of malnutrition with weight changes in each BMI group.