Original Article

A cross-sectional study on the relationship between nutrient/food intake and gut microbiota in frailty among older community residents: The Kyotango study

Yuji Naito,[1,*] Takeshi Yasuda,[2] Hiroaki Kitae,[2] Tomohisa Takagi,[2,3] Katsura Mizushima,[1] Teruhide Koyoma,[4] Ryo Inoue,[5] Norihiro Ouchi,[6,7,8] Atsuo Adachi,[6,7,8] Tadaaki Kamitani,[6,7,8] and Satoaki Matoba[6,7]

[1]Human Immunology and Nutrition Science, [2]Molecular Gastroenterology and Hepatology, [3]Department for Medical Innovation Translational Medical Science, [4]Epidemiology for Community Health and Medicine, [6]Department of Cardiovascular Medicine, and [7]Department of Longevity and Community Epidemiology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Kamigyo-ku, Kyoto 602-8566, Japan
[5]Laboratory of Animal Science, Department of Applied Biological Sciences, Faculty of Agriculture, Setsunan University, Hirakata, Japan
[8]Kyotango City Yasaka Hospital, Kyotango, Japan

(Received 18 June, 2024; Accepted 25 June, 2024; Released online in J-STAGE as advance publication 28 June, 2024)

In strategies to extend a healthy lifespan, early detection and prevention of frailty are critical. The purpose of this study was to analyze the current state and clinical risk factors of frailty among community-dwelling older to conduct a cross-sectional analysis of the individuals, correlation between frailty and nutrient intake, dietary diversity, and dietary patterns, and to elucidate the correlation between frailty-related dietary factors and the gut microbiota. The study included 786 participants aged ≥65 years from the Kyotango Multipurpose Cohort Study who had available data on their gut microbiota. Frailty was quantitatively assessed by selecting 32 items from the previously reported frailty index, with those scoring ≥0.21 classified as frailty (*n* = 119) and those with scores <0.21 as non-frailty (*n* = 667), followed by group comparisons. The frailty group had significantly higher values and rates than the non-frailty group for the following items: age, obesity (in females only), diabetes, hypertension, history of cancer treatment, polypharmacy, disturbed sleep quality, low physical activity, serum insulin levels, and high-sensitivity C-reactive protein. The frailty group had significantly lower levels of nutrients, including plant proteins, potassium (K), magnesium (Mg), iron (Fe), copper (Cu), vitamins B and C, folic acid, and total, soluble, and insoluble dietary fiber. When analyzed by food groups of dietary fiber, the frailty group had significantly lower intakes of soy products and non-green-yellow vegetables, specifically. The Japanese Diet Index score (rJDI12) was significantly lower in the frailty group, with significant deficiencies in soy products and mushrooms included in the rJDI12. Cluster analysis of the Spearman correlation values between nutrient intake related to frailty and the gut microbiota abundance revealed a positive correlation between the cluster containing dietary fiber and the abundance of the phylum Bacillota, including the [*Eubacterium*]*_eligens_*group. In conclusion, our findings clarify the current state of frailty among older community residents and suggest the importance of a diverse range of plant-based foods, including soy products and non-green yellow vegetables, through correlation analysis with nutrients and food groups, and partially reveal the involvement of the gut microbiota.

Key Words: **dietary fiber, frailty, high-sensitivity C-reactive protein, Kyotango, nutrient**

Extending healthy life expectancy is an urgent goal in the fields of medical care, nursing care, and health administration for older individuals in Japan. Frailty has garnered attention as a critical concept in achieving this extension. Frailty is a state characterized by increased vulnerability to health impairments due to various functional changes and a reduction in reserve capacity associated with aging and is considered a precursor to dependency.(1) Frailty possesses a quality of "reversibility"; it is believed that by confronting one's condition and engaging in preventive measures, the progression of frailty can be delayed, allowing individuals to return to a healthier state.

The prevention of frailty is based on three main pillars. The first is 'nutrition': ensuring adequate intake of proteins, maintaining a balanced diet, and sufficient hydration. The second is regular 'physical activity' (exercise). The third pillar is 'social participation', which involves engagement in work, leisure activities, or volunteering. Among these, ensuring adequate dietary intake of various nutrients is of paramount importance. Deficiencies in energy intake or poor nutritional quality and dietary balance have been identified as factors that contribute to frailty, with particular attention being paid to nutrient intake in older adults to ensure that their needs are met through effective nutritional guidance.

In Japan, the Ministry of Health, Labour, and Welfare has established the "Japanese Dietary Intake Standards" to promote public health and prevent lifestyle-related diseases, with revisions performed every five years. However, the relationship between dietary standards and frailty has not been sufficiently clarified. Based on these standards, it is valuable for dietitians in clinical and social practice settings to identify the nutrients that older individuals are particularly prone to lacking. The required protein intake depends on physical activity levels, and older adults require higher levels of protein intake. Nevertheless, studies distinguishing between animal and plant protein intake are sparse, and it has been highlighted that increased animal protein does not necessarily lead to frailty prevention.(2) It should be noted that not all animal proteins have the same effect in preventing frailty; for example, red meats (beef, pork, and lamb) and fish do not offer the same benefits.

Additionally, the role of the gut environment and microbiota

*To whom correspondence should be addressed.
E-mail: ynaito@koto.kpu-m.ac.jp

doi: 10.3164/jcbn.24-93

in the progression of frailty is becoming increasingly apparent, necessitating research beyond dietary guidance to explore the interactions between diet and gut microbiota. The Mediterranean diet, which has a high level of supportive evidence indicating its health benefits, is recommended for the prevention of frailty and dementia, although its effects are largely dependent on the gut microbiota and environment.[3,4] Plant-based diets are also recommended owing to their potential to prevent frailty,[5–7] and their effectiveness depends on their interactions with the gut microbiota.[4,8] However, studies on frailty, nutrient intake, and dietary consumption specifically targeting the Japanese population are scarce, particularly concerning their relationship with the gut environment. Therefore, the objective of this cross-sectional study was to investigate the current status of frailty among older residents in community settings; examine the relationship between energy intake, nutrient status, frequency of Japanese food consumption, and dietary diversity; and elucidate the correlation between nutrients related to frailty and the gut microbiota.

Materials and Methods

Study design and subjects. This study was a cross-sectional analysis conducted as part of the Kyotango Multi-purpose Cohort Study, initiated by the Department of Longevity and Community Epidemiology at Kyoto Prefectural University of Medicine. It involved 798 community-dwelling residents aged ≥65 years from the Kyotango area in northern Kyoto Prefecture who had available gut microbiota information (320 males and 478 females). This cohort study has collected approximately 2,000 data items per participant, from which the necessary items for this research were extracted. The Kyotango region, comprising two cities and two towns, has a population of just under 100,000, with over 38% of residents aged ≥65 years, making it one of the most aged regions in Kyoto Prefecture. Despite its aging population, the region is known for its high number of centenarians, with more than 200 per 100,000 individuals, marking it as one of the notable areas for longevity in Japan. The study was conducted in accordance with the Declaration of Helsinki and followed the ethical guidelines for medical research involving human subjects. The study was approved by the Ethics and Conflict of Interest Committee at Kyoto Prefectural University of Medicine (Approval No. ERB-C-885-4). Informed consent was obtained in written form from all participants.

Peripheral blood and biochemical tests. The participants in this study were part of the Kyotango Multi-purpose Cohort Study; thus, their blood biochemical data were used. Nutritional status was quantified using the controlling nutritional status (CONUT) score and Prognostic Nutritional Index (PNI).[9,10] The CONUT method calculates the total score based on albumin, total cholesterol, and lymphocyte counts, classifying the nutritional status into four levels: normal (0–1), mild (2–4), moderate (5–8), and severe (9–12) malnutrition. Each of the three parameters was scored from 0 to 3, including the score for albumin doubling. The PNI, as formulated by Onodera,[11] is calculated as follows:

$$PNI = 10 \times Albumin + 0.005 \times Total\ cholesterol$$

A PNI of 40 or less is considered indicative of malnutrition.

Assessment of dietary diversity and Japanese dietary patterns. Dietary diversity was assessed using the Dietary Variety Score (DVS). Following the method reported by Kumagai *et al.*,[12,13] scores were assigned to the consumption frequency of ten food groups (tubers, legumes, green and yellow vegetables, seaweeds, seafood, meats, eggs, milk, fruits, and fats). Points were allocated as follows: 1 point for "almost every day", and 0 points for "once every two days", "once or twice a week", or "almost never", and the total score was calculated. The Japanese dietary pattern was assessed using the rJDI12 (revised Japanese Diet Index 12), based on a report by Saji *et al.*,[14] which involved scoring the intake of 12 food items (rice, miso, seafood, green and yellow vegetables, seaweed, pickles, green tea, beef/pork, coffee, soy and soy products, fruits, and mushrooms). For all items except beef and pork, a score of +1 was assigned if the intake was above the sex-specific median. For beef and pork, a score of +1 was assigned if the intake was below the median, and the total score was computed accordingly.

Diagnosis of frailty. Frailty was diagnosed using the modified frailty index as proposed by Searle *et al.*,[15] which involves selecting 32 items out of a possible 40. Each assessment item was scored as 0 if no impairment was present and 1 if an impairment was detected. For ordinal variables, scores were adjusted to fall between 0 and 1. The total score was calculated by summing the scores and dividing them by the number of items. Following previously published research, a cut-off value of 0.21 was applied. Individuals with scores of ≥0.21 were categorized as the frailty group, and those with scores <0.21 were assigned to the non-frailty group. The frailty index was also used for quantitative analysis.

Analysis of gut microbiota. Feces collected from the participants were stored in a freezer at −80°C until DNA extraction. DNA extraction was achieved by enzymatic and bead fragmentation (NucleoSpin microbial DNA kit, Macherey-Nagel, Düren, Germany).[16] Nine variable regions (V1–V9) in the 16S rRNA provided the most useful information for phylogenetic and taxonomic studies. Among the variable regions of the 16S rRNA gene in the extracted DNA, the V3–V4 regions were amplified by PCR, and the amplified products were analyzed using MiSeq (Illumina, San Diego, CA), a next-generation sequencer, to determine the nucleotide sequence. Quantitative Insights Into Microbial Ecology 2 (QIIME 2) was used to analyze sequence data. The DADA2 model was used to correct sequencing errors, and the feature table and representative sequences were determined.[17,18] Phylogenetic diversity analysis was performed using the obtained feature information, and the types and presence ratios of bacteria constituting the flora were calculated. UniFrac distances were calculated using principal coordinate analysis, and the constituent bacteria in the samples were compared.

Statistical analysis. To examine the variation in nutrient and food intake among participants, independent of their energy intake, the residual method was used to calculate nutrient and food intake, assuming that each participant had consumed their estimated energy requirements. The residual method involves using a regression equation to determine the relationship between a group's energy intake and nutrient intake, followed by calculating the difference between an individual's energy intake and nutrient intake. This method is necessary because nutrient intake can be influenced by energy intake when examining groups. The estimated energy requirements were calculated based on the standard weight [kg, using a body mass index (BMI) of 22 kg/m^2 as the standard], basal metabolic rate (males: 21.5, females: 20.7 kcal/kg/day), and physical activity level II coefficient (ages 50–69: 1.75, ages ≥70: 1.7). Statistical analyses were performed using the JMP Pro 16 software released in 2021 by JMP Statistical Discovery LLC (SAS Institute Japan, Co. Ltd., Tokyo, Japan). Comparisons between the non-frail and frail groups were conducted using the Wilcoxon test for quantitative variables and the chi-squared test for qualitative variables. Logistic regression analysis was used to calculate odds ratios, and cluster analysis was performed based on Spearman's correlation coefficients to compare quantitative variables. All tests were considered statistically significant at $p<0.05$.

Results

Correlation between frailty frequency and background factors. For the analysis, 798 participants were included, sub-

doi: 10.3164/jcbn.24-93

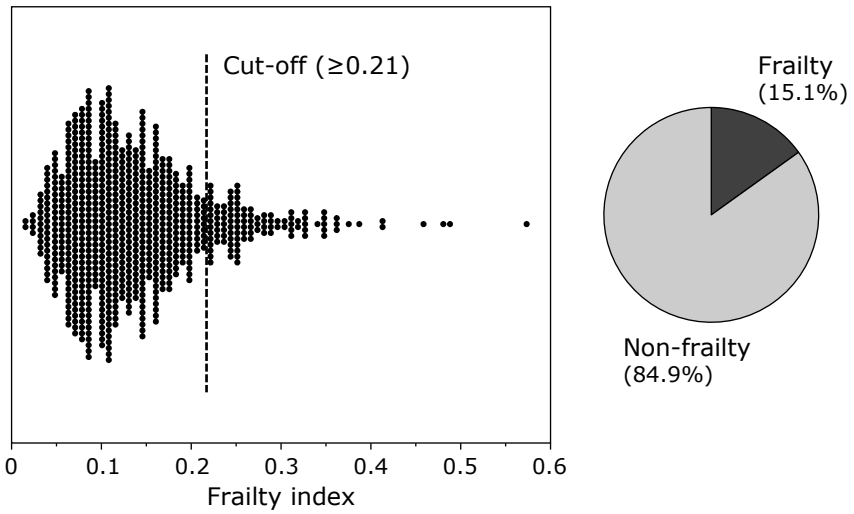

Fig. 1. Frequency of frailty evaluated by frailty index (n = 786).

sequently excluding 6 with missing frailty index data and 6 with either excessive or insufficient energy intake (<600 kcal/day or >4,000 kcal/day). Thus, 786 individuals were included in the final analysis. The distribution of the frailty index among these participants is shown in Fig. 1. Most participants had an index <0.21, indicating a relatively healthy elderly population. Of the 786 participants, 119 (15.1%) were diagnosed with frailty [49 males (15.5%) and 70 females (14.9%)]. Table 1 presents a comparison of the physical and clinical information between the non-frailty (n = 667) and frailty (n = 119) groups. The frailty group was significantly older than the non-frailty group. Sex-based differences in waist circumference and BMI were detected. Although there was no difference in BMI in males, the waist circumference was significantly higher in the frailty group than in the non-frailty group. Considering female participants, the frailty group had significantly higher waist circumference and BMI than the non-frailty group. Figure 2 presents a significant positive correlation between BMI and quantitative frailty index in females (R^2 = 0.094, p<0.001).

The frailty group had a significantly higher prevalence of diabetes, hypertension, and a history of cancer treatment than the non-frailty group. Polypharmacy, defined as the use of four or more medications, was significantly higher in the frailty group than in the non-frailty group. Although there was no difference in sleep duration, sleep quality was significantly lower in the frailty group than in the non-frailty group. Additionally, regular physical activity was significantly lower in the frailty group than in the non-frailty group. There were no significant differences in smoking history, alcohol consumption, educational level, presence of constipation, or number of cohabiting family members between the two groups. Logistic regression analysis, using frailty status as the dependent variable and various factors as independent variables, yielded a significant regression equation (χ^2 = 107.6, p<0.0001). The odds ratios for each factor related to frailty are presented in Table 2.

Differences in blood and biochemical tests between the frailty and non-frailty groups. Considering the biochemical tests, all average values were within the normal range for both groups (Table 3). The frailty group exhibited significantly lower levels of low-density and high-density lipoprotein cholesterol than the non-frailty group. Serum insulin and high-sensitivity C-reactive protein (hsCRP) levels were significantly higher in the frailty group than in the non-frailty group. According to the correlation analysis of all 786 participants, hsCRP was positively correlated with the quantitative frailty index (R^2 = 0.006, p = 0.024) (Fig. 3). Regarding nutritional indicators, the PNI values of the non-frailty and frailty groups were 42.9 ± 2.6 and 42.6 ± 3.0, respectively, with no significant difference. However, the proportion of participants with a CONUT score indicating malnutrition (score ≥2) was significantly higher in the frailty group (21.0%) than in the non-frail group (10.2%) (p<0.01, χ^2 test).

Differences in nutrient intake between the frailty and non-frailty groups. Table 4 presents the energy-adjusted nutrient intake values calculated using the residual method, including energy, protein-fat-carbohydrate (PFC) balance, proteins, fats, carbohydrates, minerals, vitamins, and dietary fibers. There were no significant differences in energy intake between the two groups. While there were no significant differences in the carbohydrate and fat percentages in the PFC balance, the percentage of protein was significantly lower in the frailty group than in the non-frailty group. Total protein intake was significantly lower in the frailty group, particularly plant protein intake, not animal protein. The frailty group had significantly lower protein intake from specific food groups, such as tubers, beans, and non-green-yellow vegetables, than the non-frailty group (Table 5). Specifically, soy and soy product intake in the frailty group was 66% of that in the non-frailty group (Fig. 4). There were no significant differences in the intakes of total fat, animal fat, plant fat, saturated fat, omega-3 and -6 fatty acids, or carbohydrates between the two groups. Although there were no differences in sodium mineral intake between the groups, potassium intake was significantly lower in the frailty group than in the non-frailty group, resulting in a significantly higher Na/K ratio. Upon comparing food groups containing potassium, no differences in fruit intake were detected; however, the intake of grains, potatoes, beans, and non-green-yellow vegetables was significantly lower in the frailty group than in the non-frailty group (Supplemental Table 1*).

The intakes of other minerals, including magnesium, iron, and copper, were significantly lower in the frailty group than in the non-frailty group. The frailty group also had a significantly lower vitamin B intake (B1, B2, B6, and B12) than the non-frailty group. The intake of both insoluble and soluble dietary fiber was significantly lower in the frailty group. Upon assessing dietary fiber intake by 15 food groups, non-green-yellow vegetables, grains, green-yellow vegetables, fruits, beans, and potetoes had the highest contribution rate (Supplemental Fig. 1*). The intake of beans and non-green-yellow vegetables, which contribute to

*See online. https://doi.org/10.3164/jcbn.24-93

Table 1. Characteristics of the non-frailty and frailty groups in older adults

	Non-frailty (*n* = 667)	Frailty (*n* = 119)	*p* value
Frailty index1	0.11 (0.08, 0.15)	0.25 (0.23, 0.30)	<0.001
Age (years)[1]	71 (69, 75)	76 (70, 81)	<0.001
Sex, *n* (%)[2]			
Male	266 (40.2)	49 (41.2)	
Female	399 (59.8)	70 (58.8)	
Waist circumference (cm)[1]			
Male	86.0 (81.0, 90.8)	87.0 (83.0, 94.0)	<0.05
Female	82.0 (76.0, 89.0)	87.6 (81.3, 94.7)	<0.001
BMI (kg/m^2)[1]			
Male	23.4 (21.9, 25.4)	24.3 (21.8, 26.1)	ns
Female	22.4 (20.7, 24.5)	24.6 (22.6, 26.8)	<0.001
Life-style related diseases, *n* (%)[2]			
Diabetes mellitus	57 (8.6)	24 (20.2)	<0.0001
Hypertention	213 (31.9)	75 (63.3)	<0.0001
Dyslipidemia	173 (25.9)	39 (31.8)	0.122
Past history of cancer	56 (8.4)	10 (16.0)	<0.01
Medication, *n* (%)[2]			
≥4	24 (3.6)	24 (20.2)	<0.0001
<4	643 (96.4)	95 (79.8)	
Smoking status, *n* (%)[2]			
Never	494 (74.2)	86 (72.3)	0.663
Former/current	172 (25.8)	33 (27.3)	
Drinking status, *n* (%)[2]			
Never	399 (60.1)	75 (63.6)	0.477
Former/current	265 (39.9)	43 (36.4)	
Education levels, *n* (%)[2]			
Primary school~high school	481 (72.1)	94 (79.0)	0.119
Junior college or higher	186 (27.9)	25 (21.00	
Sleep disorder, *n* (%)[2]			
Decrease in sleep quality	118 (17.7)	48 (40.3)	<0.001
Sleep time disorder	264 (39.8)	56 (47.1)	0.139
Constipation, *n* (%)[2]	224 (33.6)	48 (40.34)	0.154
Family living together, *n* (%)[2]			
≥2	588 (88.2)	101 (84.9)	0.316
<2	79 (11.8)	18 (15.1)	
Partner, *n* (%)[2]			
Yes	519 (77.9)	86 (72.3)	0.176
No	147 (22.1)	33 (27.3)	
Daily physical activities, *n* (%)[2]			
Yes	388 (58.2)	56 (47.1)	<0.05
No	279 (41.8)	63 (52.9)	

Data are shown as *n* (%) or median (25th, 75th percentile). [1]Wilcoxson test, [2]chi-square test.

dietary fiber intake, was significantly lower in the frailty group than in the non-frailty group (Table 6).

Differences in dietary intake diversity scores and Japanese diet scores between the frailty and non-frailty groups. Although there were no significant differences in the DVS between the groups, the rJDI12 was significantly lower in the frailty group than in the non-frailty group (Table 7). Comparing the intake of the 12 foods comprising the rJDI12, the intake of soy and soy products, and mushrooms was significantly lower in the frailty group than in the non-frailty group.

Differences in gut microbiota between the frailty and non-frailty groups. There were no significant differences in alpha diversity, beta diversity, or phylum-level occupancy rates between the two groups. Cluster analysis of the correlation between nutrients related to frailty and the abundance of top 30 genera and 7 phyla showed that dietary fiber, potassium, magnesium, and vitamin B6 were classified in the same cluster group. Figure 5 illustrates a negative correlation with the gut microbiota in cluster 1 and a positive correlation with cluster 4. All microbes in cluster 4 belonged to the phylum Firmicutes (Bacillota), including the [*Eubacterium*]_*eligens*_group, Christensenellaceae_R-7_group, and Oscillospiraceae UCG-002. The abundance of the [*Eubacterium*]*eligens*_group showed a significant positive correlation with total dietary fiber intake (Supplemental Fig. 2*).

Multivariate correlation analysis with the frailty index. A multivariate correlation analysis was performed on significant differences in blood biochemical tests and nutrient intake

*See online. https://doi.org/10.3164/jcbn.24-93

doi: 10.3164/jcbn.24-93

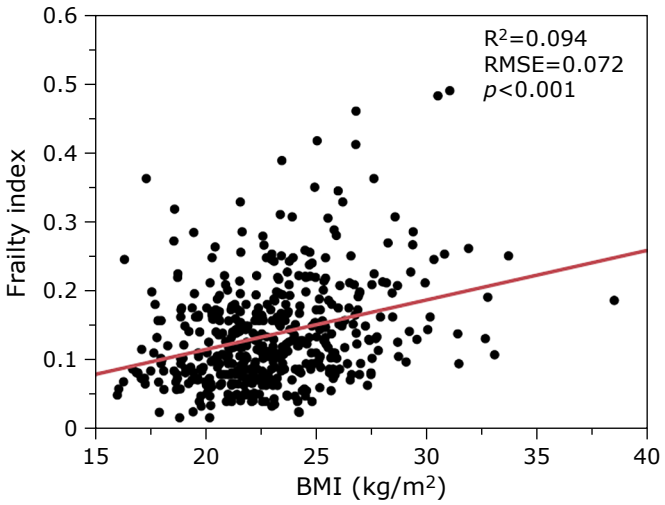

Fig. 2. Association between frailty index and body mass index (BMI) in female subjects (n = 469) RMSE, root mean squared error.

between the non-frailty and frailty groups, as well as specific gut bacteria, eliciting Spearman's rank correlation coefficients. The network diagram in Fig. 6 illustrates the presence of a positive correlation between the frailty index and serum levels of interleukin (IL)-6, hsCRP, and the Na/K ratio and a negative correlation with plant protein, vitamin B6, and total dietary fiber intake. Although there was no significant correlation between the frailty index and the occupancy rates of the gut bacteria analyzed, a strong positive correlation was detected between the occupancy rates of the three bacteria investigated and the intake of total dietary fiber and vitamin B6.

Discussion

This study was a cross-sectional study involving 786 older community residents, which explored the associations between frailty and clinical background factors, inadequate nutrient and food intake within the frailty group, and correlations between nutrient/food intake and gut microbiota. There was no significant difference in the prevalence of frailty between genders, with 15% of both men and women diagnosed as frail. Logistic regression analysis identified age, BMI, diabetes, hypertension, history of cancer treatment, polypharmacy, and low physical activity as risk factors for frailty. One distinct outcome of this study was a clear sex-based difference in the association between obesity and frailty. Weight loss is one of the five primary criteria in frailty screening. However, only females in the frailty group had significantly higher waist circumference and BMI values than those in the non-frailty group, with a significant positive correlation between BMI values and the quantitative frailty index. Although the importance of malnutrition in the pathology of frailty has been highlighted, this study did not observe weight loss, low BMI, or decreased serum protein or albumin levels in the frailty group; instead, a higher incidence of diabetes and hypertension was noted. Unlike studies focusing on outpatients or specific diseases, this community-based study clearly demonstrated differences from the control group. Upon analyzing 7,191 Japanese community residents, Watanabe *et al.*[19] reported that not only the low-BMI group but also the high-BMI group experienced higher incidences of frailty, and the high-BMI group also had higher mortality rates.[20] Although prospective cohort studies are required to clarify the causal relationship between obesity and frailty, measures to prevent obesity are likely to contribute to the prevention of frailty.

This study conducted sleep surveys using the Pittsburgh Sleep Quality Index, which found no difference in sleep duration but poorer sleep quality in the frailty group. While many previous studies have noted a correlation between sleep duration and frailty, recent reports have suggested an increasing trend in the relationship between sleep quality and frailty.[21–23] Although deficiencies in amino acids such as cysteine, proline, and serine have been reported as dietary factors linking sleep disorders and frailty, detailed information is lacking.[24] The Kyotango Longevity Cohort Study, in addition to dietary surveys using the brief self-administrative dietary habit questionnaire (BDHQ), also performs measurements such as serum amino acid profiles, potentially enabling future analyses of their relation to sleep.

Considering biochemical tests, the frailty group had significantly higher insulin and hsCRP levels than the non-frailty group, with a positive correlation with the quantitative frailty

Table 2. Odds ratio determined by logistic regression analysis for frailty

Variables		OR	Lower limit 95%	Upper limit 95%	likelihood ratio χ^2	p value
Age	75–84 vs 65–74	1.97	1.20	3.28		0.008
	85– vs 65–74	8.07	3.24	20.06	21.65	<0.0001
BMI	≥25 vs <18.5	2.53	0.57	11.13		ns
	≥25 vs 18.5–25	4.07	1.18	3.68	6.59	0.011
Diabetes mellitus		3.31	1.78	6.17	13.19	0.0002
Hypertension		3.10	1.91	5.04	21.58	<0.0001
Dyslipidemia		0.73	0.43	1.26	1.29	ns
Past history of cancer therapy		2.10	1.08	4.06	4.55	0.033
Polypharmacy		2.20	1.01	4.79	3.90	0.047
Smoking		0.80	0.45	1.42	0.58	ns
Alcohol		0.90	0.54	1/50	0.16	ns
Academic career		0.92	0.54	1.59	0.08	ns
Sleep quality		3.49	2.15	5.69	24.62	<0.0001
Sleep time		0.91	0.57	1.46	0.14	ns
Constipation		1.10	0.69	1.76	0.17	ns
Number of housemate ≥2 vs <2		0.86	0.38	1.97	0.12	ns
Spouse		1.01	0.53	1.96	0.00	ns
Daily exercise		0.57	0.36	0.91	5.58	0.0187

Table 3. Blood examination of the non-frailty and frailty groups in older adults

	Non-frailty (n = 667)	Frailty (n = 119)	p value
Biochemistry			
Total protein (g/dl)	7.2 ± 0.4	7.2 ± 0.4	ns
Albumin (g/dl)	4.2 ± 0.3	4.2 ± 0.3	ns
AST (U/L)	24 ± 8	25 ± 13	ns
ALT (U/L)	21 ± 10	22 ± 15	ns
ALP (U/L)	234 ± 68	241 ± 63	ns
γ-GTP (U/L)	31 ± 39	37 ± 56	ns
LDH (U/L)	199 ± 32	200 ± 38	ns
Total cholesterol (mg/dl)	212 ± 36	194 ± 33	<0.001
LDL (mg/dl)	127 ± 31	112 ± 29	<0.001
HDL (mg/dl)	68 ± 17	64 ± 16	<0.05
TG (mg/dl)	119 ± 60	129 ± 68	ns
DHA (μmol/L)	160 ± 42	156 ± 44	ns
EPA (μmol/L)	93 ± 45	87 ± 47	ns
EPA/AA ratio	0.47 ± 0.24	0.47 ± 0.29	ns
BUN (mg/dl)	17 ± 4	18 ± 5	ns
Creatinine (mg/dl)	0.74 ± 0.21	0.80 ± 0.25	<0.05
Uric acid (mg/dl)	4.9 ± 0.1	5.1 ± 0.1	ns
Na (mEq/L)	140 ± 2	140 ± 2	ns
K (mEq/L)	4.1 ± 0.3	4.2 ± 0.3	<0.05
BS (mg/dl)	103 ± 14	105 ± 17	ns
HbA1c (%)	5.7 ± 0.5	5.9 ± 0.8	<0.05
Peripheral blood cells			
WBC ($\times 10^3$/μl)	5.9 ± 1.4	6.1 ± 1.5	ns
RBC ($\times 10^4$/μl)	432 ± 42	427 ± 60	ns
PLT ($\times 10^4$/μl)	22.1 ± 4.9	21.9 ± 4.9	ns
%Lympho (%)	34.1 ± 7.8	33.0 ± 8.2	ns
N/L ratio	1.81 ± 0.82	1.93 ± 0.81	ns
Ferritin (ng/ml)	127 ± 100	123 ± 127	<0.05
Hormone/Vitamins			
DHEA-S (μg/dl)	98 ± 62	87 ± 52	ns
Insulin (μU/ml)	6.2 ± 4.6	7.3 ± 4.7	<0.001
Cortisol (pg/ml)	8.2 ± 2.7	7.9 ± 2.9	ns
ACTH (pg/ml)	28 ± 15	25 ± 13	ns
Adiponectin (μg/ml)	12.8 ± 7.3	13.4 ± 7.9	ns
25OH Vit. D3 (ng/ml)	22.7 ± 7.3	23.6 ± 8.7	ns
Amino acids			
Valine (μM)	223 ± 39	226 ± 38	ns
Leucine (μM)	113 ± 20	112 ± 21	ns
Isoleucine (μM)	61 ± 13	62 ± 13	ns
Taurine (μM)	53 ± 23	62 ± 82	ns
Fischer ratio	3.2 ± 0.5	3.2 ± 0.6	ns
Inflammation			
IL-6 (pg/ml)	2.1 ± 5.2	1.9 ± 1.5	<0.001
HS-CRP (mg/dl)	0.08 ± 0.15	0.10 ± 0.11	<0.05
8-OH-dG (ng/ml)	7.3 ± 3.9	7.4 ± 3.4	ns

Data are shown as mean ± SD.

index. Although insulin levels are known to correlate with obesity, elevated hsCRP levels in the frailty group suggest a link between chronic inflammation and frailty, which is an interesting outcome. Previous cohort studies have demonstrated the usefulness of hsCRP as a biomarker for inflammaging and frailty.[25–27] Moreover, in a prospective cohort study of community residents, Son *et al.*[28] revealed that groups with a lower intake of anti-inflammatory foods, as measured using the energy-adjusted dietary inflammatory index, had elevated levels of serum hsCRP and an increased frailty incidence. Although a strong positive correlation was detected between hsCRP and IL-6 in this study, IL-6 was not a better biomarker than hsCRP for the diagnosis of frailty. Furthermore, hsCRP may better assess mild chronic inflammation; however, further investigations are required.

Recent reports have frequently highlighted the association between frailty and conditions such as anemia,[29] hypoalbu-

doi: 10.3164/jcbn.24-93

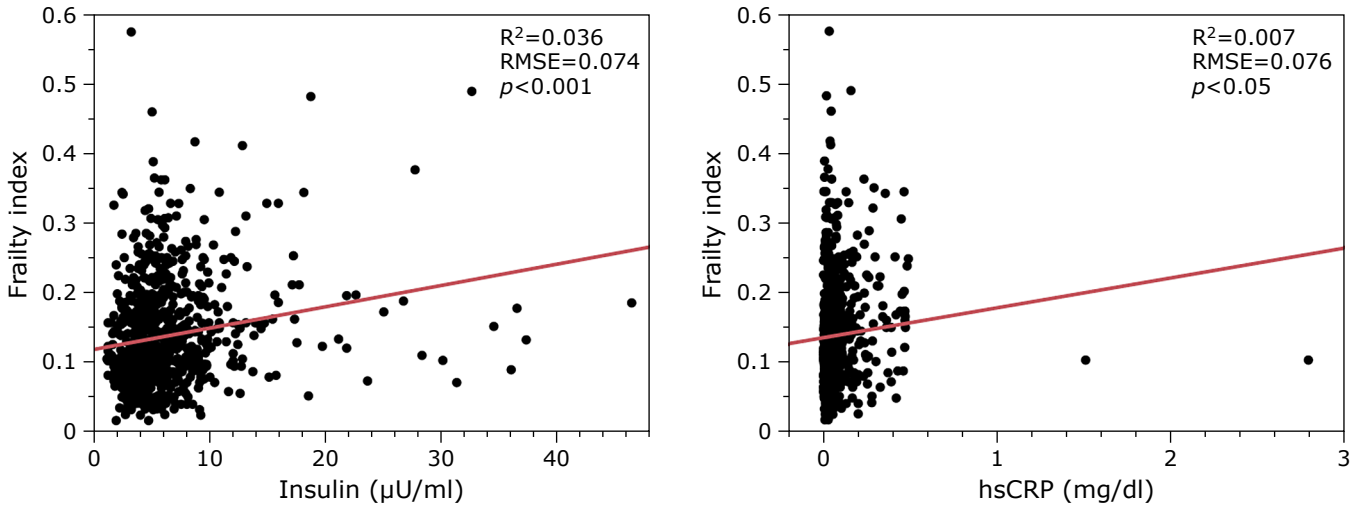

Fig. 3. Association between frailty index and serum levels of insulin or hsCRP.

minemia,[30] and vitamin D deficiency;[31] however, no significant differences were detected in the current study. It is well recognized that insufficient serum 25(OH)D3 levels in older individuals can lead to various musculoskeletal disorders, including osteoporosis and falls/fractures. However, chronic serum 25(OH)D3 insufficiency has also been noted in young females and older individuals in Japan. For instance, a report on vitamin D status among 9,084 Japanese adults showed that if the cutoff for serum 25(OH)D3 levels was set at 75 nmol/L (30 ng/ml), only 9.1% met this criterion,[32] and application of the same standard to the 786 individuals in the current trial, only 16.8% of participants would have sufficient levels. While it is intriguing that vitamin D levels were insufficient, even in a study of healthy, long-lived community residents, further research on vitamin D levels and frailty remains a crucial issue for future investigation.

One of the most notable findings of this study was the considerable difference in nutrient intake between the non-frailty and frailty groups, as quantified from the BDHQ data. In the analysis of macronutrient intake, there were no differences in fat or carbohydrate intake between the two groups. However, protein intake was significantly lower in the frailty group, both in terms of total and plant protein. Previous studies have demonstrated that low protein intake is a risk factor for frailty in older individuals, suggesting the importance of adequate protein intake after adjusting for physical activity and age to prevent frailty.[33] Cross-sectional studies of older Japanese community residents have also shown a lower incidence of frailty in groups with high protein intake,[34] indicating that an optimal level of protein intake exists.[35] However, in a cohort study of healthy older twins, Ni Lochlainn *et al.*[2] revealed that a high-protein diet in older individuals could paradoxically be a risk factor for sarcopenia, which correlates with the frailty index. Furthermore, the results of The Prospective Nurses' Health Study (NHS) cohort involving 121,700 individuals also indicated that a higher intake of plant proteins during midlife leads to an extended healthy lifespan.[36] Likewise, the current study suggests that plant protein intake, rather than animal protein intake, may prevent frailty, highlighting potatoes, soy, soy products, and non-green-yellow vegetables as notable sources of plant protein. For frailty prevention, it is crucial to gather data not only on whether the protein is animal- or plant-based but also on which food sources are crucial protein sources.

A growing number of reports have linked vitamin intake to frailty. In a prospective study of 9,030 healthy individuals (average age, 47 years), Jayanama *et al.*[37] revealed that the frailty index was significantly higher when the intake of vitamins B6, folate, and vitamin C failed to meet the recommended levels. Studies targeting older individuals have also reported low intakes of vitamins and carotenoids. Although vitamin deficiencies are expected to be less common in Japan's dietary environment, recent surveys targeting working-age individuals have shown a relatively high frequency of vitamin deficiencies, and a comprehensive deficiency in nutrients was found to be related to minor mental and physical health complaints.[38] In the current study, we also detected deficiencies in vitamin B, primarily folate and vitamin C, in the frailty group. Upon analyzing food groups containing vitamin B6, legumes and non-green yellow vegetables were identified as deficient in the frailty group (Supplemental Table 1*). The potential involvement of vitamin B6 in inhibiting inflammatory signaling[39] and inducing antioxidant enzymes may play a role in suppressing frailty, although further investigations in disease models and humans are needed.

The Na/K ratio is an indicator of the balance between sodium and potassium intake, with a high value indicating high or low sodium intake. Numerous studies have linked the Na/K ratio to cardiovascular diseases, particularly hypertension. Furthermore, several cohort studies have shown that the Na/K ratio can impact overall mortality.[40] The notable prevalence of hypertension in the frailty group and the substantially lower Na/K ratio calculated from dietary surveys in this study are intriguing findings. Additionally, the lower Na/K ratio detected in the frailty group was not due to sodium intake but rather due to poor potassium intake, suggesting an insufficiency of potassium-containing food groups. Cross-sectional and retrospective studies targeting community residents have indicated that adequate potassium intake could mitigate the severity of frailty.[41,42] Specifically, in the frailty group, the reduced intake of potassium-containing food groups was not a reduction in fruit intake but rather a reduced consumption of soy and soy products. This finding is of particular interest when considering the relationship between frailty and minerals, warranting further investigation.

Dietary fiber intake can suppress overall mortality and reduce the incidence of diabetes, heart disease, and colorectal cancer, as evidenced by systematic reviews and meta-analyses.[43] In a Japan Public Health Center (JPHC) study targeting Japanese individuals, associations between dietary fiber intake and the risks of overall death, cancer death, and cardiovascular disease death were reported,[44] emphasizing the role of dietary fiber in a nutritional approach to healthy longevity. Although direct evidence

*See online. https://doi.org/10.3164/jcbn.24-93

Table 4. Nutrients intakes of the non-frailty and frailty groups in older adults

	Non-frailty (*n* = 667)	Frailty (*n* = 119)	*p* value
Energy (kcal/day)[1]	1,800 (1,485, 2,205)	1,840 (1,506, 2,221)	0.943
Protein (g/day)[2]	76.7 (68.1, 86.7)	74.6 (67.3, 82.9)	<0.05
%PFC-protein	0.162 (0.143, 0.184)	0.156 (0.137, 0.176)	<0.05
Animal protein (g/day)[2]	46.0 (36.6, 55.2)	44.2 (35.4, 51.91)	0.159
Plant protein (g/day)[2]	30.9 (27.8, 34.0)	29.2 (26.3, 33.0)	<0.01
Lipid (g/day)[2]	54.6 (46.9, 62.1)	55.7 (47.5, 62.0)	0.976
%PFC-lipid	0.260 (0.222, 0.300)	0.268 (0.229, 0.297)	0.992
Animal lipid (g/day)[2]	26.1 (21.3, 31.4)	25.0 (20.0, 31.9)	0.646
Plant lipid (g/day)[2]	28.6 (23.2, 32.9)	28.1 (23.6, 33.0)	0.929
Saturated fatty acid (g/day)[2]	14.4 (12.0, 16.9)	14.4 (12.1, 17.0)	0.992
n-6 Fatty acid (g/day)[2]	10.1 (8.6, 11.9)	10.2 (8.6, 11.6)	0.850
n-3 Fatty acid (g/day)[2]	2.97 (2.40, 3.58)	2.92 (2.32, 3.42)	0.290
Carbohydrate (g/day)[2]	250.6 (226.8, 275.5)	254.3 (237.0, 271.3)	0.260
%PFC-carbohydrate	0.570 (0.520, 0.630)	0.570 (0.540, 0.620)	0.422
Minerals			
Na (mg/day)[2]	4,548 (3,989, 5,180)	4,541 (4,042, 5,212)	0.739
K (mg/day)[2]	2,913 (2,428, 3,394)	2,645 (2,176, 3,066)	<0.001
K/Na ratio	0.63 (0.54, 0.74)	0.57 (0.48, 0.69)	<0.001
Ca (mg/day)[2]	643 (518, 759)	603 (501, 723)	0.085
Mg (mg/day)[2]	284 (249, 325)	263 (229, 298)	<0.001
P (mg/day)[2]	1,185 (1,043, 1,340)	1,140 (983, 1,269)	<0.05
Fe (mg/day)[2]	8.71 (7.49, 10.17)	8.04 (6.78, 9.23)	<0.001
Zn (mg/day)[2]	8.66 (7.95, 9.43)	8.51 (7.75, 9.31)	0.069
Cu (mg/day)[2]	1.25 (1.12, 1.39)	1.18 (1.02, 1.32)	<0.001
Mn (mg/day)[2]	3.23 (2.65, 3.99)	3.01 (2.55, 4.04)	0.256
Vitamins			
Vit. A (μgRAE/day)[2]	781 (569, 1,062)	770 (501, 1,016)	0.366
Vit. D (μg/day)[2]	19.3 (13.4, 25.7)	18.8 (12.5, 24.2)	0.222
Vit. E (mg/day)[2,3]	7.80 (6.44, 9.10)	7.55 (6.41, 8.89)	0.220
Vit. K (mg/day)[2]	297 (213, 422)	253 (166, 370)	<0.001
Vit. B1 (mg/day)[2]	0.83 (0.72, 0.95)	0.80 (0.67, 0.89)	<0.05
Vit. B2 (mg/day)[2]	1.45 (1.22, 1.70)	1.36 (1.13, 1.56)	<0.01
Niacin (mg/day)[2]	19.5 (16.5, 22.6)	18.1 (15.2, 20.6)	<0.01
Vit. B6 (mg/day)[2]	1.45 (1.22, 1.68)	1.30 (1.10, 1.55)	<0.001
Vit. B12 (μg/day)[2]	12.8 (9.0, 16.3)	11.7 (8.2, 14.5)	<0.05
Folic acid (μg/day)[2]	371 (297, 460)	338 (270, 411)	<0.001
Pantothenic acid (mg/day)[2]	7.11 (6.21, 8.11)	6.66 (5.86, 7.59)	<0.01
Vit. C (mg/day)[2]	127.1 (92.4, 169.6)	114.9 (80.2, 143.7)	<0.01
Dietary fiber (DF)			
Total DF (g/day)[2]	13.1 (11.0, 15.8)	11.7 (9.8, 14.5)	<0.001
Total DF (g/day)[3]	13.6 (11.2, 16.5)	12.1 (10.1, 15.2)	<0.001
Soluble DF (g/day)[2]	3.3 (2.6, 4.0)	2.9 (2.2, 3.6)	<0.001
Soluble DF (g/day)[3]	3.5 (2.7, 4.2)	3.0 (2.3, 3.8)	<0.01
Insoluble DF (g/day)[2]	9.3 (7.8, 11.2)	8.4 (7.2, 10.2)	<0.001
Insoluble DF (g/day)[3]	9.7 (8.1, 11.9)	8.7 (7.6, 10.7)	<0.01

[1]Estimated by BDHQ, [2]Energy-adjusted value using residual method, [3]Energy-adjusted value using density method. Data are shown as median (25th, 75th percentile). *P* values indicated by Wilcoxon test.

linking dietary fiber intake to frailty is insufficient, the presence of chronic inflammation in the progression of frailty has been clarified, highlighting the importance of the gut barrier as an inducer of chronic inflammation.[45] The interaction between sufficient dietary fiber and the gut microbiome is increasingly considered critical for maintaining the gut barrier. Herein, we found that the total dietary fiber intake, both soluble and insoluble, was significantly lower in the frailty group than in the non-frailty group, revealing deficiencies in legumes and non-green-yellow vegetables as sources of dietary fiber. Although legumes are known as a high-protein plant source, they also contain high levels of dietary fiber, vitamin B group, and magnesium, making them a crucial ingredient for preventing frailty.

Regarding dietary patterns and frailty, there is substantial evidence suggesting that the Mediterranean diet could prevent frailty, although research on this topic in Japan is in its infancy. Cross-sectional studies on the relationship between the DVS,[46] diet quality score,[47] dietary balance scores,[48] and Japanese diet

doi: 10.3164/jcbn.24-93

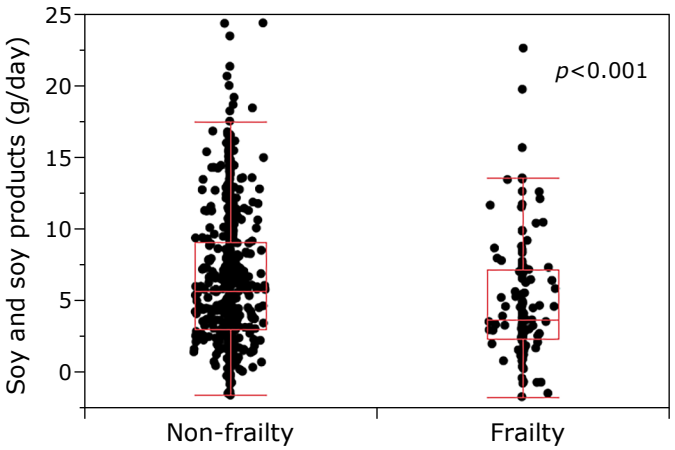

Fig. 4. Differences in soy and soy products intake between non-frailty and frailty groups.

score[49] and the incidence of frailty have been reported. The Mediterranean diet was found to be the most healthy diet in several cohort studies, including cardiovascular prevention. A one-year Mediterranean diet intervention trial involving 612 healthy individuals and pre-frailty subjects from five European countries was conducted.[3] The authors found that changes in the gut microbiome abundance due to the Mediterranean diet correlated positively with reductions in frailty markers and improvements in cognitive function and negatively with inflammation markers such as CRP and IL-17. Meta-analyses targeting older individuals have also revealed that a Mediterranean diet can reduce the risk of frailty.[50] However, the suitability of the Mediterranean diet for Japanese individuals has not been thoroughly examined. Saji *et al.*[14] calculated the Japanese dietary index (JDI) using dietary intake questionnaires. The traditional Japanese diet score was calculated based on the intake of nine items (rice, miso, seafood, green and yellow vegetables, seaweed, pickles, green tea, beef, and pork), with three items (soy and soy products, fruits, and mushrooms) added to modify the Japanese diet score, rJDI12. The authors found that the high rJDI12 group included several individuals without dementia, and some blood concentrations of gut microbiome metabolites were low. Shimizu *et al.*[51] compared the rates of grip strength decline (an indicator of sarcopenia) between individuals consuming Japanese and Mediterranean diets, revealing that groups with a higher Japanese diet (rJDI12) score had a lower odds ratio of grip strength decline, with no significant association detected between the Mediterranean diet score and the rate of individuals meeting the criteria for grip strength decline. In the current study, the Japanese diet score, rJDI12, was significantly lower in the frailty group than in the non-frailty group, and the evaluation of 12 items comprising that score revealed that the frailty group consumed substantially lower amounts of legumes, soy products, and mushrooms. These results suggest that recommending a Japanese diet pattern is a reasonable frailty prevention strategy among Japanese individuals.

Numerous reports have highlighted the academic field of gut microbiome and frailty, which has attracted considerable attention. The diversity and composition of the gut microbiome in older patients with frailty differ substantially from those of healthy older individuals.[52,53] While some cross-sectional study results indicate a reduction in alpha diversity in the frailty group, others found no such change, and most studies do not agree with these results at the genus or species levels. In the present study, we detected no significant difference in alpha diversity, as measured by the Chao-1 and Shannon indices, between the non-frailty and frailty groups. At this stage, the lack of prospective cohort studies with frailty as an endpoint and analysis of environmental factors, including diet and medications that substantially affect the gut microbiome, are considered problematic, warranting more detailed research in the future.

Recently, a Mediterranean diet intervention in five European countries showed the potential to improve frailty by regulating the gut microbiome.[3] A one-year Mediterranean diet increased the populations of anti-inflammatory bacteria, such as *Faecalibacterium prausnitzii*, Roseburia (*Roseburia hominis*), and Eubacterium [*Eubacterium rectale*, *Eubacterium eligens* (*E. eligens*), and *Eubacterium xylanophilum*], which correlated positively with a reduction in frailty markers and improvements in cognitive function, and negatively with inflammatory markers such as CRP and IL-17. In the current study, the population of butyrate-producing bacteria was maintained in the non-frailty group, and their occupancy rates positively correlated with dietary fiber intake, suggesting that butyrate-producing bacteria

Table 5. Protein intakes of the non-frailty and frailty groups in older adults

Foods	Non-frailty (n = 667)	Frailty (n = 119)	p value
Grains (g/day)	13.39 (10.91, 15.41)	13.06 (10.68, 15.14)	ns
Potatoes (g/day)	1.02 (0.57, 1.68)	0.87 (0.46, 1.21)	<0.05
Beans (g/day)	5.58 (3.04, 9.03)	3.67 (2.28, 7.07)	<0.001
Green and yellow vegetables (g/day)	1.23 (0.85, 1.84)	1.24 (0.75, 1.74)	ns
Non-green-yellow vegetables (g/day)	2.06 (1.50, 2.90)	1.75 (1.22, 2.35)	<0.001
Fruits (g/day)	0.74 (0.40, 1.24)	0.72 (0.32, 1.10)	ns
Sugar/sweet drink (g/day)	0	0	
Seafoods (g/day)	21.56 (15.04, 29.84)	20.95 (14.52, 26.75)	ns
Meats (g/day)	11.92 (8.68, 15.66)	11.76 (7.48, 14.86)	ns
Eggs (g/day)	5.12 (3.27, 7.50)	5.21 (2.84, 7.48)	ns
Milk (g/day)	5.17 (2.56, 6.84)	4.9 (2.34, 7.1)	ns
Oils and fats (g/day)	0	0	
Confectionery (g/day)	2.86 (1.68, 4.49)	2.66 (1.54, 4.42)	ns
Luxury drinks (g/day)	1.27 (0.83, 1.73)	1.21 (0.69, 1.68)	ns
Seasonings/spices (g/day)	0.98 (0.68, 1.52)	1.06 (0.73, 1.59)	ns

Each data are estimated by BDHQ/energy-adjusted value using residual method. Data are shown as median (25th, 75th percentile). *P* values indicated by Wilcoxon test.

Table 6. Dietary fiber intakes of the non-frailty and frailty groups in older adults

Foods	Non-frailty (*n* = 667)	Frailty (*n* = 119)	*p* value
Grains (g/day)	2.50 (1.80, 3.05)	2.71 (2.02, 3.34)	0.108
Potatoes (g/day)	0.81 (0.35, 1.42)	0.79 (0.36, 1.00)	0.154
Beans (g/day)	0.68 (0.29, 1.72)	0.39 (0.14, 1.39)	<0.001
Green and yellow vegetables (g/day)	2.07 (1.30, 3.31)	2.08 (1.22, 3.25)	0.646
Non-green-yellow vegetables (g/day)	3.35 (2.20, 4.87)	2.98 (1.98, 3.92)	<0.01
Fruits (g/day)	1.24 (0.62, 2.10)	1.06 (0.56, 1.78)	0.071
Sugar/sweet drink (g/day)	0	0	
Seafoods (g/day)	0	0	
Meats (g/day)	0	0	
Eggs (g/day)	0	0	
Milk (g/day)	0	0	
Oils and fats (g/day)	0	0	
Confectionery (g/day)	0.48 (0.23, 0.86)	0.48 (0.22, 0.83)	0.622
Luxury drinks (g/day)	0	0	
Seasonings/spices (g/day)	0.31 (0.17, 0.54)	0.33 (0.17, 0.66)	0.401

Each data are estimated by BDHQ/energy-adjusted value using residual method. Data are shown as median (25th, 75th percentile). *P* values indicated by Wilcoxon test.

Table 7. rJDI12 sore, DVS, and MDVS of the non-frailty and frailty groups in older adults

	Non-frailty (*n* = 667)	Frailty (*n* = 119)	*p* value
rJDI12[1]	6.82 ± 2.57	6.27 ± 2.59	<0.05
Rice (g/day)[2]	270 (208, 390)	260 (165, 429)	0.425
Miso-soup (g/day)[2]	120 (69, 216)	132 (66, 264)	0.401
Seaweed (g/day)[2]	12.3 (4.9, 24.6)	11.1 (4.4, 22.2)	0.227
Pickles (g/day)[2]	17.7 (6.1, 29.4)	20.9 (5.9, 35.5)	0.339
Green leafy vegetables (g/day)[2]	28.8 (11.5, 63.2)	28.8 (6.8, 57.5)	0.188
Seafood (g/day)[2]	85.9 (52.7, 130.5)	81.4 (43.6, 127.6)	0.247
Green tea[2]	173 (25, 433)	150 (12, 150)	0.186
Red meat + ham[2]	37.4 (21.1, 48.0)	33.9 (18.3, 45.7)	0.137
Beans[2]	72.8 (38.4, 109.1)	45.5 (31.4, 88.7)	<0.001
Fruits[2]	43.6 (24.3, 69.8)	35.5 (18.4, 63.6)	0.065
Coffee[2]	173 (150, 375)	173 (107, 433)	0.165
Mushroon[2]	10.7 (4.3, 20.5)	9.2 (2.7, 13.0)	<0.05
DVS[1]	5.07 ± 2.62	4.70 ± 2.52	0.148
mDVS[1]	5.58 ± 2.91	5.13 + 2.83	0.126

Each data are estimated by BDHQ/energy-adjusted value using residual method. Data are shown as mean ± SD[1] or median (25th, 75th percentile)[2]. *P* values indicated by Wilcoxon test.

exert anti-inflammatory effects. Notably, *E. eligens*, which not only produces butyrate but also promotes the production of anti-inflammatory cytokine IL-10, is an anti-inflammatory bacterium and one of the 15 good bacteria identified in the PREDICT1 study.[54] Recent systematic reviews have also reported lower occupancy rates of Eubacterium species in patients with frailty.[52] This study has clarified that the presence or absence of frailty and the frailty index is influenced by age, obesity, exercise, and medications. In the future, investigations into the pathogenesis of frailty and prevention strategies based on diet and the gut microbiome must consider patient background factors.

This study has several limitations. First, the frequency of food or food group intake was based on self-administered questionnaires, which may include recall bias. Second, the study was limited to relatively healthy and long-lived rural Japanese residents and may not be directly applicable to urban areas or other regions. Analysis of the gut microbiome was performed at the genus level; species-level and metabolite analyses have not yet been conducted. Therefore, considering these limitations, large-scale studies involving multiple regions are necessary in the future, and prospective cohort studies are required to clarify the potential of diverse plant-based diets and Japanese dietary patterns in reducing the risk of frailty with more definitive evidence. Fortunately, since the Kyotango Cohort Study, which forms the basis of this research, began in 2017, results from the 10-year cohort are expected in a few years, and we look forward to these findings.

Conclusion

Herein, we elucidated the current status of frailty among older residents in the well-known, long-lived region of Kyotango. The findings identified obesity, hypertension, polypharmacy, low physical activity, and chronic inflammation as significant risk factors for frailty. The importance of certain nutrients and foods was highlighted through correlation analysis of nutrients and

doi: 10.3164/jcbn.24-93

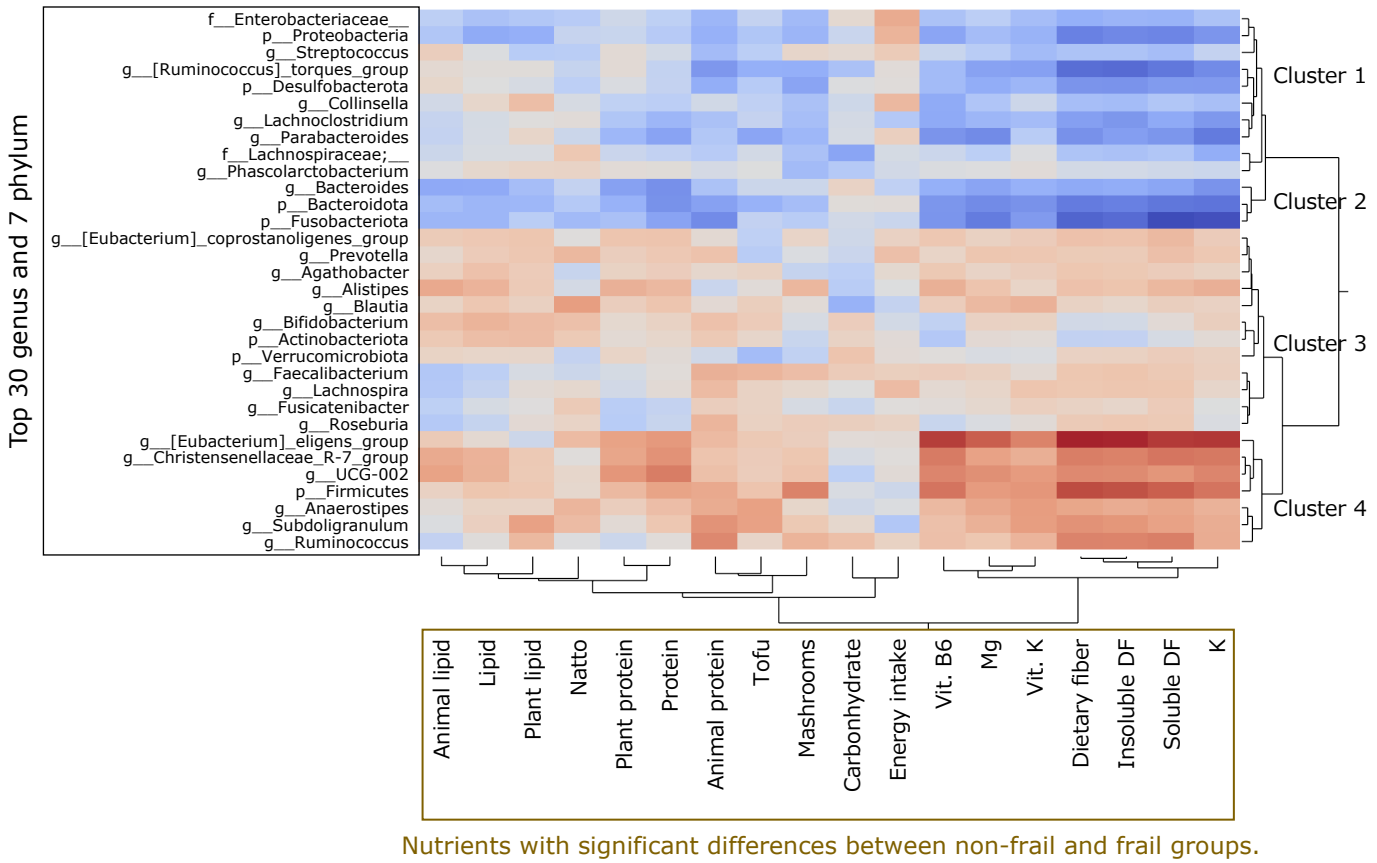

Fig. 5. Cluster analysis of the relationship between food/nutrients and the abundance of gut microbiota. See color figure in the on-line version.

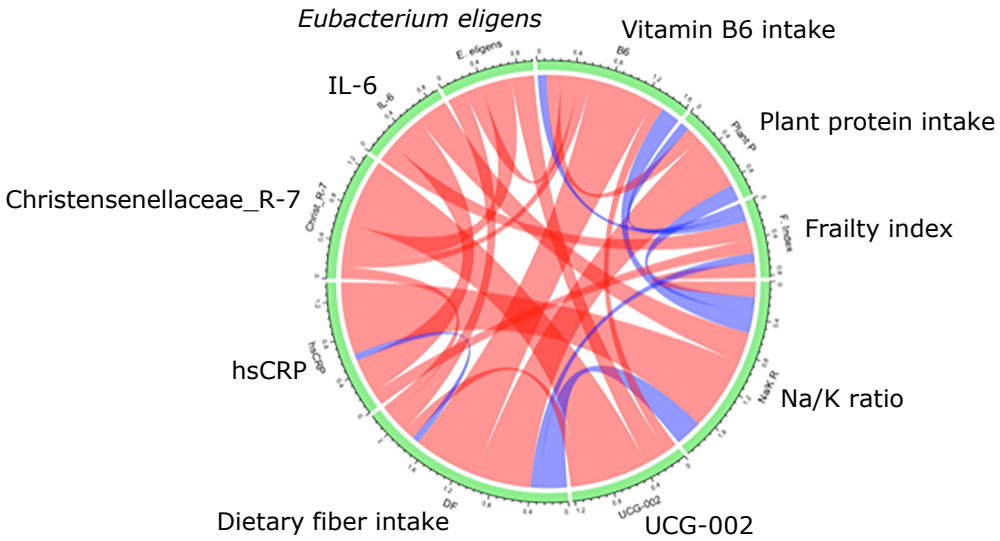

Fig. 6. Spearman rank correlation coefficient using multi-item correlation analysis. See color figure in the on-line version.

food groups with frailty. Specifically, plant-based proteins, dietary fiber, and vitamin B were identified as beneficial nutrients, whereas soy and soy products, non-green yellow vegetables, and a variety of plant-derived foods could substantially mitigate frailty. Furthermore, this study partially elucidates the relationship between these nutrients, foods, and the gut microbiota.

Acknowledgments

This work was partly supported by MAFF Commissioned project study on "Project for the realization of foods and dietary habits to extend healthy life expectancy" allotted to YN (Grant Number JPJ009842). The authors would like to thank all the participants of the Iwaki Health Promotion Project (COI-NEXT, JST

Grant Number JPMJPF2210). We would like to thank Editage (www.editage.jp) for English language editing.

Conflict of Interest

YN received scholarship funds from Taiyo Kagaku Co. Ltd., Morinaga Co. Ltd., Miyarisan Pharma Co. Ltd., Morishita-Jintan Co. Ltd., Fujikko Co. Ltd., Mizkan Co. Ltd., a collaboration research fund from Taiyo Kagaku Co., Ltd.; and received lecture fees by Takeda Pharma. Co. Ltd., Mochida Pharma. Co. Ltd., Biofermin. Co. Ltd., Otsuka Pharma. Co. Ltd., and Miyarisan Pharma. Co. Ltd. TT and RI received a collaboration research fund from PreMedica Inc. The present research was partly supported by these funds. Neither the funding agency nor any outside organization has participated in the study design or have any competing interests. These companies have approved the final version of the manuscript.

References

1 Doody P, Lord JM, Greig CA, Whittaker AC. Frailty: pathophysiology, theoretical and operational definition(s), impact, prevalence, management and prevention, in an increasingly economically developed and ageing world. *Gerontology* 2023; **69**: 927–945.
2 Ni Lochlainn M, Bowyer RCE, Welch AA, Whelan K, Steves CJ. Higher dietary protein intake is associated with sarcopenia in older British twins. *Age Ageing* 2023; **52**: afad018.
3 Ghosh TS, Rampelli S, Jeffery IB, *et al*. Mediterranean diet intervention alters the gut microbiome in older people reducing frailty and improving health status: the NU-AGE 1-year dietary intervention across five European countries. *Gut* 2020; **69**: 1218–1228.
4 Ticinesi A, Nouvenne A, Cerundolo N, Parise A, Mena P, Meschi T. The interaction between Mediterranean diet and intestinal microbiome: relevance for preventive strategies against frailty in older individuals. *Aging Clin Exp Res* 2024; **36**: 58.
5 Qi R, Yang Y, Sheng B, Li H, Zhang X. Plant-based diet indices and their association with frailty in older adults: a CLHLS-based cohort study. *Nutrients* 2023; **15**: 5120.
6 Duan Y, Qi Q, Gao T, Du J, Zhang M, Liu H. Plant-based diet and risk of frailty in older Chinese adults. *J Nutr Health Aging* 2023; **27**: 371–377.
7 Maroto-Rodriguez J, Ortolá R, García-Esquinas E, Kales SN, Rodríguez-Artalejo F, Sotos-Prieto M. Quality of plant-based diets and frailty incidence: a prospective analysis of UK biobank participants. *Age and Ageing* 2024; **53**: afae092.
8 Dominguez LJ, Veronese N, Barbagallo M. Dietary patterns and healthy or unhealthy aging. *Gerontology* 2024; **70**: 15–36.
9 Yagi T, Oshita Y, Okano I, *et al*. Controlling nutritional status score predicts postoperative complications after hip fracture surgery. *BMC Geriatr* 2020; **20**: 243.
10 Misirlioglu NF, Uzun N, Ozen GD, *et al*. The relationship between neutrophil-lymphocyte ratios with nutritional status, risk of nutritional indices, prognostic nutritional indices and morbidity in patients with ischemic stroke. *Nutrients* 2024; **16**: 1225.
11 Onodera T, Goseki N, Kosaki G. Prognostic nutritional index in gastrointestinal surgery of malnourished cancer patients. *Nihon Geka Gakkai Zasshi* 1984; **85**: 1001–1005. (in Japanese)
12 Kumagai S, Watanabe S, Shibata H, *et al*. Effects of dietary variety on declines in high-level functional capacity in elderly people living in a community. *Nihon Koshu Eisei Zasshi* 2003; **50**: 1117–1124. (in Japanese)
13 Kwon J, Suzuki T, Kumagai S, Shinkai S, Yukawa H. Risk factors for dietary variety decline among Japanese elderly in a rural community: a 8-year follow-up study from TMIG-LISA. *Eur J Clin Nutr* 2006; **60**: 305–311.
14 Saji N, Tsuduki T, Murotani K, *et al*. Relationship between the Japanese-style diet, gut microbiota, and dementia: a cross-sectional study. *Nutrition* 2022; **94**: 111524.
15 Searle SD, Mitnitski A, Gahbauer EA, Gill TM, Rockwood K. A standard procedure for creating a frailty index. *BMC Geriatr* 2008; **8**: 24.
16 Costea PI, Munch R, Coelho LP, Paoli L, Sunagawa S, Bork P. metaSNV: a tool for metagenomic strain level analysis. *PLoS One* 2017; **12**: e0182392.
17 Bolyen E, Rideout JR, Dillon MR, *et al*. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* 2019; **37**: 852–857.
18 Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016; **13**: 581–583.
19 Watanabe D, Yoshida T, Watanabe Y, Yamada Y, Kimura M; Kyoto-Kameoka Study Group. A U-shaped relationship between the prevalence of frailty and body mass index in community-dwelling Japanese older adults: the Kyoto-Kameoka Study. *J Clin Med* 2020; **9**: 1367.
20 Watanabe D, Yoshida T, Watanabe Y, Yamada Y, Miyachi M, Kimura M. Frailty modifies the association of body mass index with mortality among older adults: Kyoto-Kameoka study. *Clin Nutr* 2024; **43**: 494–502.
21 Aditi, Singh SK, Jaiswal AK, Verma M. Is there a ubiquitous association between sleep disorder and frailty? Findings from LASI (2017–18). *BMC Geriatr* 2023; **23**: 429.
22 Deng Z, Hu Y, Duan L, *et al*. Causality between sleep traits and the risk of frailty: a Mendelian randomization study. *Front Public Health* 2024; **12**: 1381482.
23 Ribeiro BC, de Athayde Costa E Silva A, de Souza LBR, de Araújo Moraes JB, Carneiro SR, Neves LMT. Risk stratification for frailty, impairment and assessment of sleep disorders in community-dwelling older adults. *Exp Gerontol* 2024; **187**: 112370.
24 Kinoshita K, Otsuka R, Takada M, *et al*. Dietary amino acid intake and sleep duration are additively involved in future cognitive decline in Japanese adults aged 60 years or over: a community-based longitudinal study. *BMC Geriatr* 2023; **23**: 653.
25 Cheng Z, He D, Li J, Wu Q, Liu Z, Zhu Y. C-reactive protein and white blood cell are associated with frailty progression: a longitudinal study. *Immun Ageing* 2022; **19**: 29.
26 van Sleen Y, Shetty SA, van der Heiden M, *et al*. Frailty is related to serum inflammageing markers: results from the VITAL study. *Immun Ageing* 2023; **20**: 68.
27 Luo YF, Cheng ZJ, Wang YF, *et al*. Unraveling the relationship between high-sensitivity C-reactive protein and frailty: evidence from longitudinal cohort study and genetic analysis. *BMC Geriatr* 2024; **24**: 222.
28 Son BK, Lyu W, Tanaka T, Yoshizawa Y, Akishita M, Iijima K. Impact of the anti-inflammatory diet on serum high-sensitivity C-Reactive protein and new-onset frailty in community-dwelling older adults: a 7-year follow-up of the Kashiwa cohort study. *Geriatr Gerontol Int* 2024; **24 Suppl 1**: 189–195.
29 Murata S, Ebeling M, Meyer AC, Schmidt-Mende K, Hammar N, Modig K. Blood biomarker profiles and exceptional longevity: comparison of centenarians and non-centenarians in a 35-year follow-up of the Swedish AMORIS cohort. *Geroscience* 2024; **46**: 1693–1702.
30 Corti MC, Guralnik JM, Salive ME, Sorkin JD. Serum albumin level and physical disability as predictors of mortality in older persons. *JAMA* 1994; **272**: 1036–1042.
31 Okada M, Hama Y, Futatsuya R, *et al*. Association between masticatory performance, nutritional intake, and frailty in Japanese older adults. *Nutrients* 2023; **15**: 5075.
32 Miyamoto H, Kawakami D, Hanafusa N, *et al*. Determination of a serum 25-hydroxyvitamin D reference ranges in Japanese adults using fully automated liquid chromatography-tandem mass spectrometry. *J Nutr* 2023; **153**: 1253–1264.
33 Coelho-Júnior HJ, Rodrigues B, Uchida M, Marzetti E. Low protein intake is associated with frailty in older adults: a systematic review and meta-analysis of observational studies. *Nutrients* 2018; **10**: 1334.
34 Nanri H, Yamada Y, Yoshida T, *et al*. Sex difference in the association between protein intake and frailty: assessed using the kihon checklist indexes among older adults. *J Am Med Dir Assoc* 2018; **19**: 801–805.
35 Nanri H, Watanabe D, Yoshida T, *et al*. Adequate protein intake on comprehensive frailty in older adults: Kyoto-Kameoka Study. *J Nutr Health Aging* 2022; **26**: 161–168.
36 Ardisson Korat AV, Shea MK, Jacques PF, *et al*. Dietary protein intake in midlife in relation to healthy aging—results from the prospective Nurses'

doi: 10.3164/jcbn.24-93

Health Study cohort. *Am J Clin Nutr* 2024; **119**: 271–282.
37 Jayanama K, Theou O, Blodgett JM, Cahill L, Rockwood K. Frailty, nutrition-related parameters, and mortality across the adult age spectrum. *BMC Med* 2018; **16**: 188.
38 Kagami-Katsuyama H, Sato-Ueshima M, Satoh K, *et al*. The relationship between mental and physical minor health complaints and the intake of dietary nutrients. *Nutrients* 2023; **15**: 865.
39 Zhang P, Tsuchiya K, Kinoshita T, *et al*. Vitamin B6 prevents IL-1β protein production by inhibiting NLRP3 inflammasome activation. *J Biol Chem* 2016; **291**: 24517–24527.
40 Okayama A, Okuda N, Miura K, *et al*. Dietary sodium-to-potassium ratio as a risk factor for stroke, cardiovascular disease and all-cause mortality in Japan: the NIPPON DATA80 cohort study. *BMJ Open* 2016; **6**: e011632.
41 Kaimoto K, Yamashita M, Suzuki T, *et al*. Association of protein and magnesium intake with prevalence of prefrailty and frailty in community-dwelling older Japanese women. *J Nutr Sci Vitaminol (Tokyo)* 2021; **67**: 39–47.
42 Otsuka R, Zhang S, Tange C, *et al*. Association of dietary intake with the transitions of frailty among Japanese community-dwelling older adults. *J Frailty Aging* 2022; **11**: 26–32.
43 Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet* 2019; **393**: 434–445.
44 Katagiri R, Goto A, Sawada N, *et al*. Dietary fiber intake and total and cause-specific mortality: the Japan Public Health Center-based prospective study. *Am J Clin Nutr* 2020; **111**: 1027–1035.
45 Naito Y. Gut frailty: its concept and pathogeneis. *Digestion* 2024; **105**: 49–57.
46 Hayakawa M, Motokawa K, Mikami Y, *et al*. Low dietary variety and diabetes mellitus are associated with frailty among community-dwelling older Japanese adults: a cross-sectional study. *Nutrients* 2021; **13**: 641.
47 Watanabe D, Kurotani K, Yoshida T, *et al*. Diet quality and physical or comprehensive frailty among older adults. *Eur J Nutr* 2022; **61**: 2451–2462.
48 Nakamoto M, Nagashima T, Tanaka Y, *et al*. Validation of a dietary balance score in middle-aged and older community-dwelling Japanese. *J Med Invest* 2023; **70**: 377–387.
49 Shimizu A, Okada K, Tomata Y, Uno C, Kawase F, Momosaki R. Association between Japanese diet adherence and muscle weakness in Japanese adults aged ≥50 years: findings from the JSTAR cohort study. *Int J Environ Res Public Health* 2023; **20**: 7065.
50 Dominguez LJ, Donat-Vargas C, Sayon-Orea C, *et al*. Rationale of the association between Mediterranean diet and the risk of frailty in older adults and systematic review and meta-analysis. *Exp Gerontol* 2023; **177**: 112180.
51 Shimizu A, Okada K, Tomata Y, Uno C, Kawase F, Momosaki R. Association of Japanese and mediterranean dietary patterns with muscle weakness in Japanese community-dwelling middle-aged and older adults: post hoc cross-sectional analysis. *Int J Environ Res Public Health* 2022; **19**: 12636.
52 Rashidah NH, Lim SM, Neoh CF, *et al*. Differential gut microbiota and intestinal permeability between frail and healthy older adults: a systematic review. *Ageing Res Rev* 2022; **82**: 101744.
53 Wang XM, Fan L, Meng CC, *et al*. Gut microbiota influence frailty syndrome in older adults: mechanisms and therapeutic strategies. *Biogerontology* 2024; **25**: 107–129.
54 Mukherjee A, Lordan C, Ross RP, Cotter PD. Gut microbes from the phylogenetically diverse genus *Eubacterium* and their various contributions to gut health. *Gut Microbes* 2020; **12**: 1802866.