BRILL WAGENINGEN ACADEMIC

BENEFICIAL MICROBES 15 (2024) 449–463

Beneficial Microbes
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RESEARCH ARTICLE

Effects of fermented milk intake and physical activity on the suppression of age-related decline in physical fitness among the elderly

K. Shimamoto[1], *R. Amamoto*[1], *S. Park*[2], *T. Suwa*[1], *H. Makino*[1], *S. Matsubara*[1] *and Y. Aoyagi*[2]*

[1]Food Research Department, Yakult Central Institute, 5-11 Izumi, Kunitachi-shi, Tokyo 186-8650, Japan; [2]Exercise Sciences Research Group, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakaecho, Itabashi-ku, Tokyo 173-0015, Japan; *aoyagi@tmig.or.jp

Received 23 March 2023 | Accepted 20 June 2024 | Published online 5 July 2024 | Published in issue 2 September 2024

Abstract

Physical deterioration in the elderly can lead to disability and mortality. Although the intake of fermented milk has been recently attracting attention as a proposed measure to prevent physical weakness, studies and findings are limited. Here, we investigated the effect of intake of fermented milk products on suppression of age-related decline in physical fitness through a long-term epidemiological study of community-dwelling elderly people who are capable of independent living. A retrospective analysis was conducted on 581 elderly people aged 65-92 years from the Nakanojo Study, with the addition of a 5-year prospective analysis on 240 elderlies. Subjects were arbitrarily grouped on the basis of questionnaire estimates of fermented milk products intake (<3 or ≥3 days/week) and pedometer/accelerometer-determined patterns of physical activity (<7,000 or ≥7,000 steps/day). After adjustment for potential confounders, the retrospective study showed that the group consuming fermented milk products ≥3 days/week showed significantly faster walking speeds than the <3 days/week group. The group taking ≥7,000 steps/day had a significantly faster walking speed than the group taking <7,000 steps/day. Those who did both walked the fastest, indicating an additive effect. Adding protein or energy intake as a covariate to the potential confounders found a correlation between the intake of fermented milk products and walking speed, suggesting that the effect of fermented milk products consumption is independent of nutritional intake status, due to the beneficial properties of bacteria included in fermented milk. The 5-year prospective study confirmed a clear relationship between the frequency of consumption of fermented milk products and the suppression of preferred walking speed decline. Our findings suggest that habitual intake of fermented milk contributes to the suppression of walking speed decline in elderly people.

Keywords

fermented milk intake – daily physical activity – frailty – the elderly – epidemiological study

1 Introduction

With the rapid ageing of the population, the number of elderly people is increasing worldwide. In many elderly people, a gradual decline in physiological reserve increases vulnerability to stress, leading to a state of 'frailty' (Clegg *et al.*, 2013), in which life functions tend to deteriorate, gradually leading to the need for nursing care. This intermediate stage can be restored to a healthy state through intervention. Therefore, early

Published with license by Koninklijke Brill BV | DOI: 10.1163/18762891-BJA00025
 | ISSN: 1876-2891 (online)

detection of frailty and appropriate intervention should reduce the number of elderly people in need of care (Iijima *et al.*, 2021). Frailty criteria (Clegg *et al.*, 2013; Satake and Arai, 2020) use physical fitness as indicators, such as grip strength and preferred walking speed (Cruz-Jentoft *et al.*, 2019). There is an urgent need to build preventive measures against the decline in physical fitness of the elderly.

Exercise and physical activity can reduce the decline in physical fitness of the elderly (Keevil *et al.*, 2016; Sanchez-Sanchez *et al.*, 2019; Yokozuka *et al.*, 2022). We have been conducting an epidemiological study to determine habitual physical activity patterns among elderly people aged 65 years and older living in Nakanojo Town, Gunma Prefecture, Japan (the Nakanojo Study), and shown that those who walk more than 7,000-8,000 steps per day as physical activity have faster walking speeds (Aoyagi *et al.*, 2009). Because ageing can limit exercise and physical activity, other measures are needed to prevent the decline in physical fitness in addition to daily physical activity (Shinkai *et al.*, 2016). So it would be of interest to see whether a combination of measures implemented by older adults who are already physically active would be effective in further reducing declines in physical fitness.

Several reports have suggested that the consumption of fermented milk products by the elderly may reduce the risk of frailty (Cuesta-Triana *et al.*, 2019; Siefkas *et al.*, 2023). It is also known that the bacteria contained in fermented milk, such as lactic acid bacteria and bifidobacteria, contribute to the improvement of the intestinal environment and immunomodulating effects (Pique *et al.*, 2019; Wilkins and Sequoia, 2017), and these effects have been suggested to possibly contribute to the prevention of frailty (Piggott and Tuddenham, 2020). A recent report also showed that consumption of *Lactiplantibacillus plantarum* enhanced performance in physical ability tests of elderly people in a care home (Lee *et al.*, 2021), suggesting that beneficial bacteria intake may inhibit age-related decline in physical strength. However, the available evidence on the relationship of physical fitness and fermented milk or the bacteria included in the fermented milk is limited, and the factors affecting the physical fitness indicators have not been clarified.

Here, we investigated the effect of fermented milk intake on age-related decline in physical fitness through a long-term epidemiological study of community-dwelling elderly people who are capable of independent living. We examined the relationships of the intake of fermented milk products and habitual physical activity with indices of physical fitness (preferred and maximal walking speeds, grip strength, and skeletal muscle mass index) in a retrospective epidemiological study of community-dwelling elderly people. We also analysed the combination of the intake of fermented milk products and physical activity to clarify any additive effects. Further, we conducted a 5-year prospective study to evaluate the causal relationship between the habitual consumption of fermented milk products and the suppression of the decline of physical fitness among the elderly.

2 Materials and methods

Subjects

Subjects were self-supporting Japanese aged 65 years or older who had been recruited to the Nakanojo Study (Amamoto *et al.*, 2021; Aoyagi *et al.*, 2017, 2019). The criteria for inclusion here included functional independence and the absence of chronic or progressive conditions (e.g. cancer, arthritic diseases, Parkinson's disease, Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, dementia) that could limit physical activity or have a major effect on the individual's perceived quality of life. For the retrospective study, we selected 581 subjects (224 men and 357 women) aged 65 to 92 years for whom data on frequency of intake of fermented milk products, physical fitness (preferred and maximal walking speeds, grip strength and skeletal muscle mass index), physical activity and nutrient intake could be obtained. For the prospective study, 240 subjects (103 men and 137 women) aged 65 to 91 years who participated in physical fitness testing in both 2014 and 2019 were selected.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. After the protocol, stresses, and possible risks had been fully explained to them, subjects gave their written informed consent to participate in a study approved by the ethics review committee of the Tokyo Metropolitan Institute of Gerontology.

Estimation of frequency of intake of fermented milk products

The frequency of intake of overall fermented milk products, excluding cheese, was estimated by a certified nutritionist during an interview. Photographs of 24 typical fermented milk products available in Japan were shown to the subjects as examples. Subjects were asked how many days they consumed products of this type per week over the last 5 years. As in our previous report, subjects were classed as consuming the products either <3 or ≥3 days/week (Amamoto *et al.*, 2021; Aoyagi *et al.*, 2017).

Measurement of habitual physical activity

Physical activity patterns were measured for 24 h/day for a month, using a uniaxial acceleration sensor (Lifecorder; Suzuken Co. Ltd., Nagoya, Aichi, Japan), as described previously (Aoyagi and Shephard, 2009, 2010, 2011, 2013). The average number of steps taken per day and the daily cumulative duration of moderate-intensity exercise (>3 metabolic equivalents) were calculated for each subject. In this study, subjects were classed as taking either <7,000 or ≥7,000 steps/day. Cross-sectional and/or longitudinal analyses (Aoyagi and Shephard, 2009, 2010, 2011, 2013) have shown a low prevalence or incidence of various chronic diseases and disorders (including cancer of the colon and rectum) (Aoyagi and Shephard, 2011) in elderly people undertaking daily physical activities above disease-specific thresholds.

Physical fitness testing

Preferred and maximal walking speeds were determined over a 5-m distance with the use of a stopwatch (SVAE101; Seiko Corp., Minato, Tokyo, Japan), as described previously (Aoyagi *et al.*, 2004). Subjects completed two trials to determine each walking speed as the average speed of each measurement. In 1 of 581 subjects, maximal walking speed was not measured due to the subject's request. Peak handgrip force of the dominant hand was assessed with a Smedley dynamometer (ES-100; Evernew Co. Ltd., Koto, Tokyo, Japan). Two trials were performed, and the larger of the two readings was used.

Assessment of anthropometric characteristics and blood profiles

The physical characteristics of subjects (age, sex, height, body mass, body mass index [BMI], and muscle mass) were determined by standard anthropometric techniques (Shephard *et al.*, 2013). The skeletal muscle mass index, which is skeletal muscle mass corrected for height squared, was used as a measure of muscle mass. Owing to mechanical failure, 3 of the 581 values were missing. Biochemical profiles (triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, glycosylated haemoglobin A_{1c}, blood sugar, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, γ-glutamyl transpeptidase, albumin, creatinine, uric acid, and estimated glomerular filtration rate) were measured by standard methods (Health Sciences Research Institute Inc., Yokohama, Kanagawa, Japan).

Blood pressure and bone assessment

Blood pressure was measured after 5 min of seated rest with an automatic sphygmomanometer (BP-103iII; Colin Medical Technology Co. Ltd., Komaki, Aichi, Japan). At least one further measurement was made after a further 5-min rest if the initial reading suggested that an individual had become hypertensive (or, rarely, hypotensive). The osteosonic index of the calcaneus was measured with an Achilles ultrasonic bone densitometer (AOS-100; Aloka Co. Ltd., Mitaka, Tokyo, Japan), as described previously (Shephard *et al.*, 2017).

Measurement of nutrient intake

Nutritional status was evaluated over 1 week by a nutritionist using food models, photographs, and the Food Frequency Questionnaire Based on Food Groups v. 3.5 (Kenpakusha Co. Ltd., Bunkyo, Tokyo, Japan), a 20-item questionnaire covering 29 food groups and 10 methods of food preparation. From the responses, the daily consumption of energy, nutrients, and food groups during the 1-2 months before the start of the study was estimated. The estimated nutrients were protein, lipids, carbohydrates, dietary fibre, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, cholesterol, sodium, potassium, calcium, magnesium, iron, and vitamin C.

Statistical analysis

R v. 3.3 software (https://cran.r-project.org/) was used throughout (Ihaka and Gentleman, 1996). In the retrospective study, the 581 subjects were divided (men and women separately) into groups based on the reported frequency of ingestion of fermented milk products (<3 vs ≥3 days/week) and the pattern of habitual physical activity (<7,000 vs ≥7,000 steps/day). In principle, analyses of covariance assessed independent differences between groups with respect to anthropometry, supplement consumption, physical activity, physical health, nutrition, and blood (with control for age) or with respect to physical fitness (with control for age, BMI, smoking status, and alcohol consumption) (Model 1). We also analysed independent differences between groups in physical fitness by adding the covariance of protein intake to Model 1 (Model 2) or of energy intake to Model 1 (Model 3). Chi-squared tests assessed differences in the percentages of current smokers and current alcohol consumers between groups. In the prospective study, analyses integrated men and women. Paired *t*-tests assessed differences in the 240 subjects between 2014 and 2019 with respect to physical fitness to confirm age-related decline. These subjects were divided into groups based on the reported frequency of ingestion of fermented milk products (<3 vs ≥3 days/week) at baseline (2014). Analyses of covariance assessed independent differences between groups with respect to changes in walking speed from 2014 to 2019 (with control for age, sex, BMI, smoking status, alcohol consumption, and preferred or maximal walking speeds in 2014). All statistical differences were declared at $P < 0.05$.

3 Results

Retrospective study

Characteristics of subjects consuming fermented milk products for <3 or ≥3 days/week

Of the 581 subjects, 126 of the 224 men and 254 of the 357 women consumed fermented milk products ≥3 days/week (Table 1). There were no significant differences in age, body mass or BMI between the two groups of either sex. There were also no differences in the number of steps taken or the duration of moderate-intensity activity. Men had significantly higher amounts of protein and lipids and women had significantly higher amounts of protein, lipids, and energy in the ≥3 days/week group.

Relationship between intake of fermented milk products and physical fitness indices

Men ingesting fermented milk products ≥3 days/week had significantly faster preferred and maximal walking speeds (Table 2). Women ingesting fermented milk products ≥3 days/week tended to have a higher grip strength, but there was no significant difference in grip strength or muscle mass index between the two groups of either sex (Table 2).

To investigate whether fermented milk intake affects physical fitness independently of nutritional intake, we used protein intake (Model 2) and energy intake (Model 3) as covariates. The intake of fermented milk products was correlated with walking speeds in both analyses (Table 2).

Characteristics of subjects taking steps for <7,000 or ≥7,000 steps/day

Of the 581 subjects, 108 of the 224 men and 161 of the 357 women took ≥7,000 steps/day (Table 3). Those who took <7,000 steps/day had a significantly higher age, body mass and BMI.

Relationship between physical activity and physical fitness indices

Adjusted for age, BMI, smoking status, and alcohol intake, the preferred and maximal walking speeds were significantly faster in the men and women averaging ≥7,000 steps/day (Table 4). The skeletal muscle mass index was significantly higher in the women averaging ≥7,000 steps/day. There were no significant differences in grip strength.

Effect of a combination of intake of fermented milk products and physical activity on walking speed

Men and women who both consumed fermented milk products ≥3 days/week and took ≥7,000 steps/day walked fastest (Figure 1). Men were significantly fastest and women were marginally fastest in the ≥3 days/week + ≥7,000 steps/day group than in any other group. Analyses adding protein or energy intake as a covariate showed the same results as in Model 1 (Supplementary Table S1).

Group differences in estimated age calculated from walking speed

From the slope of the regression line between preferred walking speed and age in men, the rate of decline in walking speed per year was calculated to be 0.0082 m/s (Supplementary Figure S1). The group difference of 0.06 m/s (Table 2) due to the intake of fermented milk products corresponds to 7.3 years, and the group difference of 0.11 m/s (Table 4) due to daily physical activity corresponds to 13.4 years. The difference of 0.18 m/s between the <3 days/week + <7,000 steps/day group and the ≥3 days/week + ≥7,000 steps/day group (Figure 1) corresponds to 22.0 years.

Prospective study

Characteristics of the study subjects

In 2014, 165 of the 240 people consumed fermented milk products ≥3 days/week (Table 5). Physical fitness indices reduced significantly over the 5-year period (Supplementary Table S2).

Relationship between frequency of intake of fermented milk products and 5-year change in walking speed

Analyses with additional covariates showed that the 5-year change in preferred walking speed was significantly smaller in the ≥3 days/week group (Table 6).

TABLE 1 Characteristics of subjects consuming fermented milk products <3 or ≥3 days/week during the past 5 years[1]

	Men				Women			
	<3 days/week		≥3 days/week		<3 days/week		≥3 days/week	
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Anthropometric parameters								
Age (years)	98	74.3 ± 6.8	126	75.1 ± 6.7	103	72.3 ± 6.1	254	72.7 ± 6.0
Height (m)	98	1.62 ± 0.06	126	1.63 ± 0.06*	103	1.50 ± 0.06	254	1.50 ± 0.06
Body mass (kg)	98	61.1 ± 8.5	126	61.6 ± 8.3	103	51.3 ± 8.7	254	51.3 ± 7.1
Body mass index (kg/m^2)	98	23.3 ± 2.7	126	23.1 ± 2.9	103	22.7 ± 3.3	254	22.7 ± 2.9
Physical health parameters								
Systolic blood pressure (mmHg)	84	132 ± 17	108	129 ± 17	93	125 ± 14	219	129 ± 18
Diastolic blood pressure (mmHg)	84	79 ± 11	108	77 ± 9	93	74 ± 10	219	75 ± 10
Calcaneal osteosonic index (×10^6)	98	2.50 ± 0.33	126	2.58 ± 0.36	103	2.18 ± 0.21	252	2.19 ± 0.22
Fermented milk products consumption								
Past 5 years fermented milk products (days/week)	98	0.56 ± 0.77	126	5.90 ± 1.67***	103	0.93 ± 0.84	254	6.08 ± 1.55***
Habitual physical activity patterns								
Step count (steps/day)	98	7,683 ± 4,068	126	7,234 ± 3,372	103	7,105 ± 2,890	254	7,081 ± 2,820
Duration of exercise >3 METs (min/day)	98	19.7 ± 17.6	126	17.8 ± 15.9	103	15.6 ± 11.9	254	15.3 ± 13.3
Current smoking and drinking habits								
Smoker (number [%])	98	10 [10.2]	126	10 [7.9]	103	5 [4.9]	254	4 [1.6]
Alcohol consumer (number [%])	98	63 [64.3]	126	87 [69.0]	103	23 [22.3]	254	59 [23.2]
Nutrient intakes								
Energy (kcal/day)	98	2,107 ± 544	126	2,248 ± 651	103	1,860 ± 477	254	1,989 ± 479*
Protein (g/day)	98	72.2 ± 22.1	126	81.7 ± 27.7**	103	67.6 ± 21.4	254	75.0 ± 20.9**
Lipid (g/day)	98	63.6 ± 22.4	126	73.2 ± 29.5**	103	62.8 ± 21.6	254	68.7 ± 22.3*
Carbohydrate (g/day)	98	287 ± 75	126	290 ± 87	103	249 ± 61	254	261 ± 61
Dietary fibre (g/day)	98	15.9 ± 4.8	126	17.9 ± 6.2*	103	15.2 ± 4.3	254	17.0 ± 5.0**
Saturated fatty acid (g/day)	98	19.4 ± 7.5	126	22.4 ± 9.3*	103	19.1 ± 7.1	254	21.2 ± 7.2*
Monounsaturated fatty acid (g/day)	98	21.3 ± 7.7	126	24.0 ± 10.7*	103	21.3 ± 7.9	254	22.6 ± 7.8
Polyunsaturated fatty acid (g/day)	98	13.6 ± 4.7	126	15.1 ± 6.1	103	13.3 ± 4.9	254	14.1 ± 4.6
Cholesterol (mg/day)	98	310 ± 130	126	359 ± 143*	103	310 ± 121	254	345 ± 110**
Sodium (mg/day)	98	4,903 ± 1,674	126	4,741 ± 1,854	103	4,285 ± 1,861	254	4,406 ± 1511
Potassium (mg/day)	98	2,725 ± 928	126	3,071 ± 1,066*	103	2,502 ± 794	254	2,838 ± 797***
Calcium (mg/day)	98	691 ± 254	126	827 ± 300***	103	640 ± 223	254	772 ± 234***

TABLE 1 (Continued)

	Men				Women			
	<3 days/week		≥3 days/week		<3 days/week		≥3 days/week	
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Magnesium (mg/day)	98	290 ± 88	126	320 ± 106*	103	259 ± 79	254	290 ± 80**
Iron (mg/day)	98	8.73 ± 2.88	126	10.1 ± 4.0**	103	8.17 ± 2.95	254	9.38 ± 3.12***
Vitamin C (mg/day)	98	106 ± 50	126	130 ± 58**	103	105 ± 44	254	129 ± 48***
Blood profiles								
Triglyceride (mmol/l)	82	1.46 ± 0.81	102	1.50 ± 0.83	84	1.52 ± 1.08	196	1.49 ± 0.98
High-density lipoprotein cholesterol (mmol/l)	82	1.45 ± 0.32	102	1.51 ± 0.39	84	1.72 ± 0.49	196	1.69 ± 0.39
Low-density lipoprotein cholesterol (mmol/l)	82	2.80 ± 0.71	102	2.93 ± 0.80	84	3.44 ± 0.75	196	3.18 ± 0.64**
Glycosylated haemoglobin A_{1c} (%)	82	6.00 ± 0.84	102	5.65 ± 0.42***	84	5.81 ± 0.79	196	5.66 ± 0.49
Blood sugar (mmol/l)	82	7.17 ± 2.82	102	6.20 ± 1.37**	84	6.15 ± 1.99	196	5.97 ± 1.20
Glutamic oxaloacetic transaminase (IU/l)	82	24.4 ± 8.4	102	24.1 ± 6.7	84	21.9 ± 3.6	196	22.7 ± 5.9
Glutamic pyruvic transaminase (IU/l)	82	20.8 ± 8.8	102	21.1 ± 8.5	84	17.3 ± 5.5	196	17.9 ± 6.8
γ-Glutamyl transpeptidase (IU/l)	82	31.5 ± 20.9	102	31.3 ± 20.7	84	20.8 ± 15.3	196	25.4 ± 37.8
Albumin (g/l)	81	43.4 ± 2.4	102	43.8 ± 2.5	84	44.2 ± 2.2	196	44.3 ± 2.4
Creatinine (μmol/l)	82	84.9 ± 37.5	102	83.5 ± 34.3	84	61.3 ± 10.0	196	62.7 ± 9.8
Uric acid (μmol/l)	81	316 ± 90	102	335 ± 75	84	270 ± 64	196	276 ± 57
Estimated glomerular filtration rate (ml/min/1.73 m²)	82	64.8 ± 16.3	102	64.0 ± 12.5	84	64.9 ± 11.4	196	63.0 ± 10.3

1 Values are presented as mean ± standard deviation (SD) or n (%). METs = metabolic equivalents. Intergroup differences in the ratios of smoker/non-smoker and alcohol consumer/non-consumer were assessed by χ^2 tests. Independent differences in each of the other variables between groups were assessed by analyses of covariance, after adjustment for age. Independent differences in age between groups were assessed by *t*-tests. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 versus <3 days/week.

4 Discussion and conclusion

Both retrospective and prospective studies showed that habitual intake of fermented milk products contributed to the inhibition of walking speed reduction in the elderly (Table 2 and 6), in addition to daily physical activity (Table 4) (Aoyagi *et al.*, 2009; Mijnarends *et al.*, 2016; Sanchez-Sanchez *et al.*, 2019). Walking speeds were fastest in the group that regularly consumed fermented milk products and engaged in greater physical activity (Figure 1), indicating an additive effect.

Adequate dietary and protein intake contributes to muscle mass and strength, which improves walking functions (Okada *et al.*, 2020; Scognamiglio *et al.*, 2005). Therefore, it was concern that nutritional intake status may have played a role in the effect of ageing on physical

TABLE 2 Physical fitness in subjects consuming fermented milk products <3 or ≥3 days/week during the past 5 years[1]

	<3 days/week		≥3 days/week		Model		
	n	Mean ± SD	n	Mean ± SD	1	2	3
Men							
Preferred walking speed (m/s)	98	1.31 ± 0.20	126	1.37 ± 0.21	*	*	*
Maximal walking speed (m/s)	98	2.02 ± 0.42	126	2.15 ± 0.45	**	*	*
Peak handgrip force (N)	98	346 ± 80	126	343 ± 72			
Skeletal muscle mass index (kg/m^2)	98	7.75 ± 0.91	125	7.69 ± 0.92			
Women							
Preferred walking speed (m/s)	103	1.36 ± 0.23	254	1.38 ± 0.23			
Maximal walking speed (m/s)	102	1.99 ± 0.33	254	1.98 ± 0.36			
Peak handgrip force (N)	103	215 ± 53	254	224 ± 50	#		
Skeletal muscle mass index (kg/m^2)	103	6.19 ± 0.59	252	6.25 ± 0.55			

1 Values are presented as mean ± standard deviation (SD). Independent differences in physical functions between groups were assessed by analyses of covariance, after adjustment for age, body mass index (BMI), smoking status and alcohol intake (Model 1); age, BMI, smoking status, alcohol intake and protein intake (Model 2); and age, BMI, smoking status, alcohol intake and energy intake (Model 3). #$P < 0.1$, *$P < 0.05$, **$P < 0.01$ versus <3 days/week.

function observed in this study. However, the analysis adjusted for protein or energy intake showed a positive correlation between the intake of fermented milk products and walking speed (Table 2 and Supplementary Table S1). These results imply that the suppression of the decline in physical fitness observed in this study is independent of nutritional intake status, and may be due to the beneficial properties of bacteria included in fermented milk products.

The intake of bacteria such as lactobacilli and bifidobacteria have shown to increase the production of short-chain fatty acids in the intestine (Wang *et al.*, 2014; Wullt *et al.*, 2007). Since these short-chain fatty acids are a source of energy for muscles (LeBlanc *et al.*, 2017), these increases may lead to improved physical fitness. Other studies have reported a relationship of lactobacilli intake with low blood levels of tumour necrosis factor-alpha and interleukin-6 (Huang *et al.*, 2019), which are associated with muscle weakness and the development of frailty (Tuttle *et al.*, 2020), and with an increase in endurance after exercise (Huang *et al.*, 2019). Therefore, it is possible that the anti-inflammatory effects of bacterial intake on muscles can inhibit the decline in exercise function. Moreover, it has been shown that several lactobacilli and *Lactococcus* strains enhances muscle mass and muscle strength in mice (Chen *et al.*, 2019; Chen *et al.*, 2016; Sugimura *et al.*, 2018). Together with the results of this cohort study, the findings suggest that regular intake of beneficial bacteria included in fermented milk affects the gut microbiota and environment, suppressing the decline in physical fitness during old age.

The average preferred walking speed in men was 0.06 m/s faster with fermented milk products intake of ≥3 days/week (Table 2), 0.11 m/s faster with ≥7,000 steps/day (Table 4), and 0.18 m/s faster with both (Figure 1). These differences in walking speed correspond to respective differences of 7.3, 13.4, and 22.0 years. The differences in preferred walking speed are comparable to those reported for elderly Japanese subjects who took amino acid supplements and exercise together (Kim *et al.*, 2012), suggesting that physical fitness can be further improved through the intake of fermented milk and physical activity together. In women, the preferred walking speed was faster in the group consuming fermented milk products ≥3 days/week than the <3 days/week group; however, no significant difference was observed, suggesting that there may be differences between both sexes. The positive relationship between fermented milk intake and walking speed tended to be observed in the group of 7,000 steps or more for both men and women (Figure 1, <3 days/week + ≥7,000 steps/day group vs ≥3 days/week + ≥7,000 steps/day group), suggesting that this positive relationship may be more easily observed in the group with higher physical activity levels. Men took more steps than women in this study (Table 1), suggesting that differences in the amount of daily physical activity between men and women may have influenced the sex difference in the positive relationship between fermented milk intake and walking speed.

The 5-year prospective analysis showed a progressive decline in physical fitness, with decreased walking speed, grip strength, and muscle mass (Supple-

TABLE 3 Characteristics of subjects taking <7,000 or ≥7,000 steps/day[1]

	Men				Women			
	<7,000 steps/day		≥7,000 steps/day		<7,000 steps/day		≥7,000 steps/day	
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Anthropometric parameters								
Age (years)	116	76.3 ± 7.4	108	73.1 ± 5.6 ***	196	73.8 ± 6.2	161	71.0 ± 5.4***
Height (m)	116	1.62 ± 0.06	108	1.63 ± 0.06	196	1.50 ± 0.06	161	1.51 ± 0.06
Body mass (kg)	116	62.2 ± 9.0	108	60.6 ± 7.5 **	196	51.9 ± 7.8	161	50.6 ± 7.4**
Body mass index (kg/m²)	116	23.5 ± 3.2	108	22.8 ± 2.3 **	196	23.1 ± 3.1	161	22.2 ± 2.8**
Physical health parameters								
Systolic blood pressure (mmHg)	95	129 ± 18	97	131 ± 15	165	128 ± 17	147	127 ± 17
Diastolic blood pressure (mmHg)	95	77 ± 11	97	78 ± 9	165	74 ± 10	147	75 ± 10
Calcaneal osteosonic index (×10⁶)	116	2.54 ± 0.41	108	2.56 ± 0.28	194	2.17 ± 0.22	161	2.21 ± 0.21
Fermented milk products consumption								
Past 5 years fermented milk products (days/week)	116	3.79 ± 2.94	108	3.31 ± 3.01	196	4.74 ± 2.67	161	4.42 ± 2.76
Habitual physical activity patterns								
Step count (steps/day)	116	4,630 ± 1,602	108	10,439 ± 2,828 ***	196	5,065 ± 1,362	161	9,550 ± 2,127***
Duration of exercise >3 METs (min/day)	116	8.95 ± 7.76	108	29.0 ± 17.4 ***	196	7.74 ± 5.22	161	24.7 ± 13.4***
Current smoking and drinking habits								
Smoker (number [%])	116	11 [9.5]	108	9 [8.3]	196	6 [3.1]	161	3 [1.9]
Alcohol consumer (number [%])	116	77 [66.4]	108	73 [67.6]	196	34 [17.3]	161	48 [29.8]**
Nutrient intakes								
Energy (kcal/day)	116	2,117 ± 563	108	2,261 ± 650	196	1,943 ± 477	161	1,963 ± 488
Protein (g/day)	116	76.0 ± 26.5	108	79.1 ± 25.0	196	73.4 ± 22.0	161	72.1 ± 20.4
Lipid (g/day)	116	67.6 ± 28.0	108	70.5 ± 25.8	196	67.3 ± 22.2	161	66.6 ± 22.3
Carbohydrate (g/day)	116	277 ± 73	108	302 ± 89 *	196	255 ± 61	161	261 ± 61
Dietary fibre (g/day)	116	16.6 ± 5.3	108	17.6 ± 6.1	196	16.1 ± 4.9	161	17.1 ± 4.7
Saturated fatty acid (g/day)	116	20.6 ± 8.9	108	21.7 ± 8.4	196	20.8 ± 7.1	161	20.5 ± 7.4
Monounsaturated fatty acid (g/day)	116	22.2 ± 10.5	108	23.4 ± 8.5	196	22.3 ± 7.9	161	22.2 ± 7.9
Polyunsaturated fatty acid (g/day)	116	14.1 ± 5.7	108	14.9 ± 5.4	196	13.8 ± 4.7	161	14.0 ± 4.7
Cholesterol (mg/day)	116	333 ± 142	108	343 ± 136	196	343 ± 115	161	326 ± 114
Sodium (mg/day)	116	4,715 ± 1,694	108	4,916 ± 1,862	196	4,326 ± 1,606	161	4,426 ± 1,635
Potassium (mg/day)	116	2,820 ± 926	108	3,026 ± 1,108	196	2,679 ± 805	161	2,817 ± 810
Calcium (mg/day)	116	752 ± 263	108	785 ± 313	196	734 ± 243	161	735 ± 233
Magnesium (mg/day)	116	297 ± 90	108	317 ± 108	196	276 ± 81	161	287 ± 80
Iron (mg/day)	116	9.42 ± 3.54	108	9.56 ± 3.76	196	8.98 ± 3.26	161	9.08 ± 2.94
Vitamin C (mg/day)	116	118 ± 55	108	121 ± 58	196	117 ± 46	161	128 ± 50*

TABLE 3 (Continued)

	Men				Women			
	<7,000 steps/day		≥7,000 steps/day		<7,000 steps/day		≥7,000 steps/day	
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Blood profiles								
Triglyceride (mmol/l)	92	1.49 ± 0.84	92	1.48 ± 0.80	151	1.60 ± 1.13	129	1.38 ± 0.83
High-density lipoprotein cholesterol (mmol/l)	92	1.43 ± 0.37	92	1.53 ± 0.36 *	151	1.66 ± 0.42	129	1.75 ± 0.41
Low-density lipoprotein cholesterol (mmol/l)	92	2.76 ± 0.77	92	2.99 ± 0.74	151	3.27 ± 0.71	129	3.24 ± 0.66
Glycosylated haemoglobin A_{1c} (%)	92	5.82 ± 0.72	92	5.78 ± 0.60	151	5.72 ± 0.51	129	5.69 ± 0.69
Blood sugar (mmol/l)	92	6.72 ± 2.41	92	6.55 ± 1.95	151	5.99 ± 1.21	129	6.06 ± 1.75
Glutamic oxaloacetic transaminase (IU/l)	92	24.6 ± 8.4	92	23.9 ± 6.5	151	22.2 ± 5.2	129	22.8 ± 5.4
Glutamic pyruvic transaminase (IU/l)	92	20.9 ± 9.2	92	21.0 ± 7.9	151	17.7 ± 6.6	129	17.8 ± 6.2
γ-Glutamyl transpeptidase (IU/l)	92	30.8 ± 18.5	92	31.9 ± 22.9	151	26.4 ± 38.9	129	21.3 ± 23.5
Albumin (g/l)	91	43.5 ± 2.7	92	43.8 ± 2.2	151	44.1 ± 2.4	129	44.5 ± 2.2
Creatinine (μmol/l)	92	85.8 ± 36.5	92	82.5 ± 34.8	151	62.7 ± 10.1	129	61.7 ± 9.6
Uric acid (μmol/l)	91	326 ± 87	92	328 ± 77	151	278 ± 59	129	270 ± 59
Estimated glomerular filtration rate (ml/min/1.73 m^2)	92	62.8 ± 14.2	92	66.0 ± 14.3	151	62.8 ± 10.5	129	64.5 ± 10.8

1 Values are presented as mean ± standard deviation (SD) or n (%). METs = metabolic equivalents. Intergroup differences in the ratios of smoker/non-smoker and alcohol consumer/non-consumer were assessed by χ^2 tests. Independent differences in each of the other variables between groups were assessed by analyses of covariance, after adjustment for age. Independent differences in age between groups were assessed by *t*-tests. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus <7,000 steps/day.

TABLE 4 Physical fitness in subjects taking <7,000 or ≥7,000 steps/day[1]

	<7,000 steps/day		≥7,000 steps/day	
	n	Mean ± SD	n	Mean ± SD
Men				
Preferred walking speed (m/s)	116	1.29 ± 0.22	108	1.40 ± 0.17**
Maximal walking speed (m/s)	116	1.98 ± 0.44	108	2.22 ± 0.41**
Peak handgrip force (N)	116	331 ± 74	108	359 ± 74
Skeletal muscle mass index (kg/m^2)	116	7.73 ± 0.99	107	7.70 ± 0.82
Women				
Preferred walking speed (m/s)	196	1.31 ± 0.22	161	1.46 ± 0.21***
Maximal walking speed (m/s)	195	1.89 ± 0.35	161	2.10 ± 0.32***
Peak handgrip force (N)	196	216 ± 50	161	228 ± 51
Skeletal muscle mass index (kg/m^2)	194	6.22 ± 0.54	161	6.25 ± 0.58*

1 Values are presented as mean ± standard deviation (SD). Independent differences in physical functions between groups were assessed by analyses of covariance, after adjustment for age, body mass index (BMI), smoking status and alcohol intake. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus <7,000 steps/day.

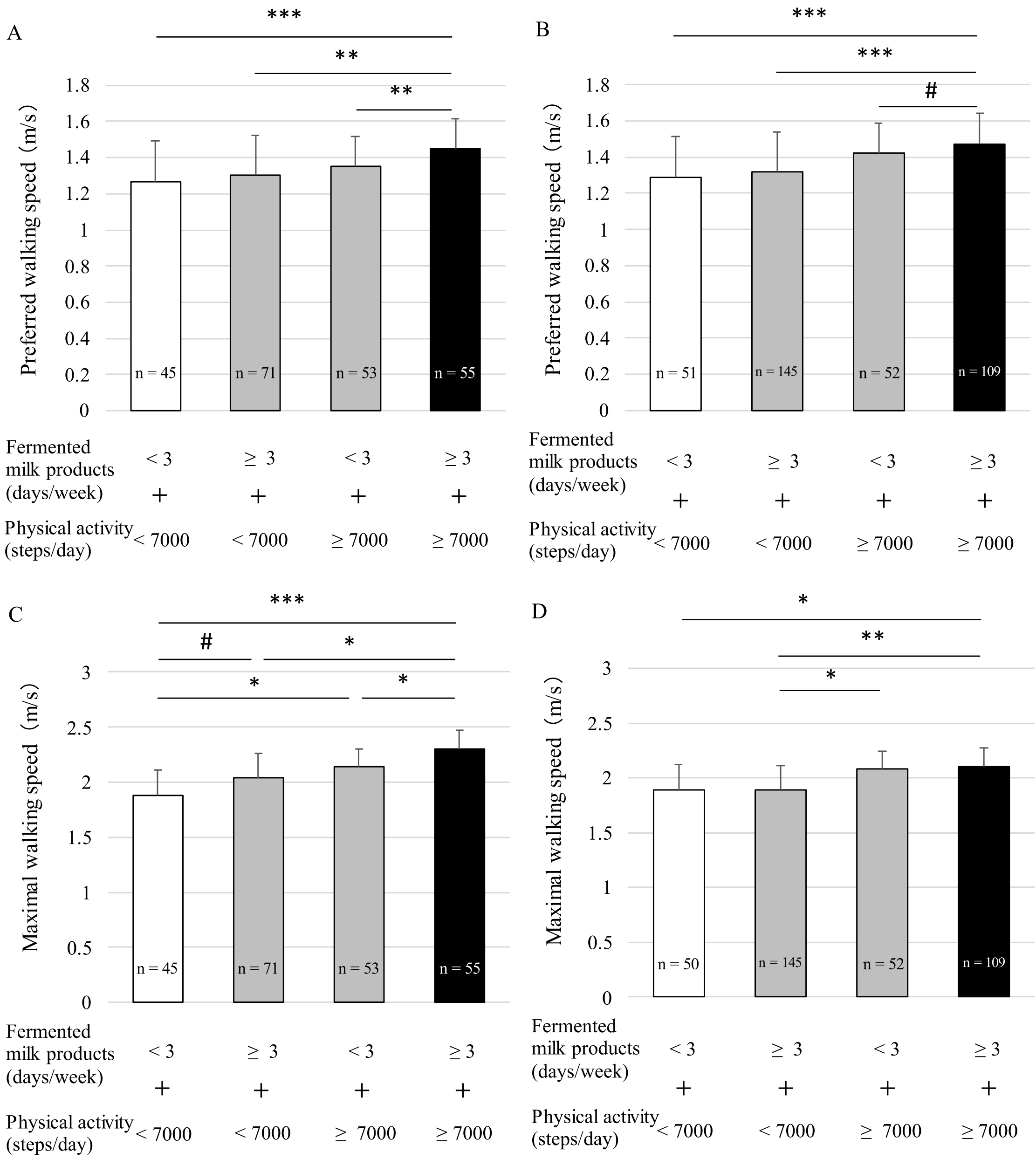

FIGURE 1 Interactions between intake of fermented milk products and habitual physical activity patterns in terms of (A) men's and (B) women's preferred walking speeds, and (C) men's and (D) women's maximal walking speeds. Data are means and standard deviations. Inter-combination differences in preferred and maximal walking speeds were assessed by analyses of covariance, after adjustment for age, body mass index (BMI), smoking status and alcohol intake. #$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

mentary Table S2). The preferred walking speed was reduced by ~0.015 m/s/year, comparable to the rate in healthy elderly subjects in Japan (Makizako *et al.*, 2017; Taniguchi *et al.*, 2016). However, the intake of fermented milk products suppressed the decline by ~40% (Table 6). These results show that we were able to evaluate the effect of the intake of fermented milk products on the age-related decline in physical fitness of healthy elderly people.

One of the limitations of this study is that this analysis cannot account for the effects of the specific ingredients or the contained bacteria, as this study did not investigate the details of the fermented milk products consumed by the subjects. In addition, the prospective survey could not take into account changes in the intake of fermented milk products during the follow-up period, because the frequency of intake after the start of follow-up was not investigated. Moreover, individuals ingesting fermented milk products ≥3 days/week or taking ≥7,000 steps/day might differ from their peers in taking a greater overall interest in personal health, embracing other facets of healthy living that could lead

TABLE 5 Characteristics of subjects consuming fermented milk products <3 or ≥3 days/week in 2014[1]

	<3 days/week		≥3 days/week	
	n	Mean ± SD	n	Mean ± SD
Anthropometric parameters				
Age (years)	75	74.4 ± 6.2	165	74.1 ± 5.4
Sex (men/women, %)	75	54.7/45.3	165	37.6/62.4*
Height (m)	75	1.57 ± 0.09	165	1.55 ± 0.09
Body mass (kg)	75	55.8 ± 8.8	165	54.6 ± 9.4
Body mass index (kg/m^2)	75	22.6 ± 2.7	165	22.7 ± 2.6
Physical health parameters				
Systolic blood pressure (mmHg)	60	126 ± 15	141	126 ± 15
Diastolic blood pressure (mmHg)	60	77 ± 11	141	75 ± 9
Calcaneal osteosonic index (×10^6)	75	2.33 ± 0.33	165	2.29 ± 0.29
Fermented milk products consumption				
Past 5 years fermented milk products (days/week)	75	0.76 ± 0.82	165	6.02 ± 1.61***
Habitual physical activity patterns				
Step count (steps/day)	56	7,556 ± 3,205	127	7,292 ± 3,216
Duration of exercise >3 METs (min/day)	56	19.2 ± 17.3	127	17.3 ± 16.9
Current smoking and drinking habits				
Smoker (number [%])	75	4 [5.3]	165	8 [4.8]
Alcohol consumer (number [%])	75	37 [49.3]	165	53 [32.1]*
Nutrient intakes				
Energy (kcal/day)	61	2,136 ± 645	146	2,178 ± 636
Protein (g/day)	61	76.1 ± 26.2	146	81.5 ± 24.7
Lipid (g/day)	61	68.4 ± 28.5	146	74.5 ± 26.1
Carbohydrate (g/day)	61	287 ± 85	146	284 ± 80
Dietary fibre (g/day)	61	17.0 ± 4.7	146	18.1 ± 5.9
Saturated fatty acid (g/day)	61	21.0 ± 9.3	146	22.9 ± 7.9
Monounsaturated fatty acid (g/day)	61	22.9 ± 9.9	146	24.3 ± 8.9
Polyunsaturated fatty acid (g/day)	61	14.6 ± 6.1	146	15.4 ± 5.8
Cholesterol (mg/day)	61	335 ± 132	146	369 ± 124
Sodium (mg/day)	61	5,061 ± 1,941	146	4,781 ± 1,658
Potassium (mg/day)	61	2,911 ± 995	146	3,044 ± 1,013
Calcium (mg/day)	61	752 ± 288	146	833 ± 274*
Magnesium (mg/day)	61	303 ± 96	146	317 ± 102
Iron (mg/day)	61	9.14 ± 3.15	146	10.3 ± 3.9*
Vitamin C (mg/day)	61	118 ± 51	146	132 ± 54
Blood profiles				
Triglyceride (mmol/l)	59	1.41 ± 0.91	139	1.55 ± 0.78
High-density lipoprotein cholesterol (mmol/l)	59	1.64 ± 0.48	139	1.60 ± 0.40
Low-density lipoprotein cholesterol (mmol/l)	59	2.98 ± 0.72	139	3.10 ± 0.69
Glycosylated haemoglobin A_{1c} (%)	59	5.72 ± 0.44	139	5.61 ± 0.46
Blood sugar (mmol/l)	59	6.29 ± 1.58	139	5.95 ± 1.27
Glutamic oxaloacetic transaminase (IU/l)	59	23.4 ± 5.1	139	23.6 ± 5.8
Glutamic pyruvic transaminase (IU/l)	59	18.6 ± 5.5	139	19.9 ± 8.1

TABLE 5 (Continued)

	<3 days/week		≥3 days/week	
	n	Mean ± SD	n	Mean ± SD
γ-Glutamyl transpeptidase (IU/l)	59	25.1 ± 14.9	139	23.1 ± 11.9
Albumin (g/l)	59	44.0 ± 2.4	139	44.5 ± 2.3
Creatinine (μmol/l)	59	76.1 ± 45.3	139	69.3 ± 15.4
Uric acid (μmol/l)	59	297 ± 86	139	294 ± 78
Estimated glomerular filtration rate (ml/min/1.73 m²)	59	65.7 ± 16.1	139	63.8 ± 11.4

[1] Values are presented as mean ± standard deviation (SD) or n (%). METs = metabolic equivalents. Intergroup differences in the ratios of men/women, smoker/non-smoker, and alcohol consumer/non-consumer were assessed by χ^2 tests. Independent differences in each of the other variables between groups were assessed by analyses of covariance, after adjustment on age for sex and the other variables for age and sex. *$P < 0.05$, ***$P < 0.001$ versus <3 days/week.

TABLE 6 Changes in walking speed from 2014 to 2019 in subjects consuming fermented milk products <3 or ≥3 days/week[1]

	<3 days/week		≥3 days/week	
	n	Mean ± SD	n	Mean ± SD
Δ Preferred walking speed (m/s)	75	−0.11 ± 0.14	165	−0.064 ± 0.169*
Δ Maximal walking speed (m/s)	75	−0.26 ± 0.24	165	−0.27 ± 0.27

[1] Values are presented as mean ± standard deviation (SD). Independent differences in changes in walking speeds between groups were assessed by analyses of covariance, after adjustment for age, sex, body mass index (BMI), smoking status, alcohol intake and preferred or maximal walking speeds in 2014. *$P < 0.05$.

to faster walking speeds. We examined several lifestyle covariates; people who ingested fermented milk products ≥3 days/week were somewhat better nourished, and those who engaged in moderate habitual daily physical activity were younger and slimmer than their peers, but in most respects, the various groups of study participants appeared to be well matched. Furthermore, we co-varied for the most important lifestyle determinants of physical fitness (age, sex, BMI, cigarette smoking, and alcohol consumption), although the statistical adjustment for these factors may have been less than complete.

Both retrospective and prospective analyses showed that preferred walking speed was correlated with the intake of fermented milk products, suggesting that fermented milk intake influences muscle endurance. On the other hand, maximal walking speed was correlated only in the retrospective study. To investigate the effect of fermented milk intake on lower-extremity instantaneous force, it will be necessary to conduct physical fitness tests.

The decline in walking speed with age in the elderly is an important issue related to falls (Landi *et al.*, 2012) and impairments in activities of daily living (Fried *et al.*, 2001; Tanimoto *et al.*, 2012), as well as the risk of death (Fried *et al.*, 2001). Future studies of the relationships of fermented milk and the beneficial bacteria intake with physical fitness, care requirements and mortality risk are expected to clarify the potential of fermented milk intake for limiting frailty and extending healthy life span and life expectancy.

Supplementary material

Supplementary material is available online at:
https://doi.org/10.6084/m9.figshare.26117011

Table S1. Interactions between intake of fermented milk products and habitual physical activity patterns in terms of walking speed, with addition of protein or energy intake as a covariate.

Table S2. Changes over 5 years in physical fitness.

Figure S1. Relationship between preferred walking speed and age in men.

Acknowledgements

We gratefully acknowledge the expert technical assistance of the research and nursing staff of the Tokyo Metropolitan Institute of Gerontology, the Yakult Central Institute, the University of Tokyo, and the Nakanojo

Public Health Center. We thank the subjects whose conscientious participation made the Nakanojo Study possible. This study was supported by grants [Grant-in-Aid for Encouragement of Young Scientists: 12770037 and Grant-in-Aid for Scientific Research (C): 15500503, (C): 17500493, (B): 19300235, and (B): 23300259] from the Japan Society for the Promotion of Science and grants from the Yakult Honsha Co., Ltd. and the Tokyo Metropolitan Institute of Gerontology.

Authors' contribution

KS and YA designed the study. KS, RA, SP, TS, HM and SM performed the study. YA acquired subjects and data, analysed the data. KS and YA drafted the manuscript; RA, SP, TS, HM and SM revised the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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