Master's Thesis of College of Medicine

Association of Change in Protein Intake and Change in Appendicular Skeletal Muscle Mass in Middle-aged Korean Women

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Abstract

Purpose

Women after middle age are more susceptible to sarcopenia than other age groups or men. In particular, sarcopenia is prevalent in middle-aged women as unbalanced inflammatory cytokines and sex hormones, during and after menopause promote muscle catabolism. Study about association of protein intake with muscle mass in middle-aged women is an important in analyzing the causes of sarcopenia and preventing it. The purpose of our study was to analyze the correlation between the change in protein intake and the change in muscle mass in middle-aged women, and to investigate other factors to mitigate muscle loss.

Methods

All data of middle-aged and elder women (over 40 years) in Korea were derived from the urban cohort in the Health Examinees study. In our study, a total of 42,094 participants was classified into 5 groups according to the increase or decrease in protein intake: <-25; -25≤, <-5; -5≤, <5; 5≤, <25; and ≥25 (g/days). Based on a prediction equation, predicted appendicular skeletal muscle mass index (ASMi) was derived. Adjusted means and 95% confidence intervals (CI) of changes in ASMi were calculated using multiple liner regression analysis.

Results

During the follow-up period, 16% of participants reduced their protein intake by more than 25g. The group whose protein intake changed by more than -5g and less than 5g was 21.1% of the study population. 23% of the study population increased their protein intake by more than 5g and less than 25g. The group whose protein intake increased by 25g, or more was the smallest group with 2,907 people, 7.4% of the total. BMI decreased with increasing changes in protein intake ($p<0.001$). Calorie intake also tended to decrease as the change in protein intake increased ($p<0.001$). The study population was divided into five groups according to change in protein intake, and the change in ASMi of each group was examined. After adjusting for potential confounders using three different models, it was found that there was a negative association between changes in protein intake and changes in ASMi, with the magnitude of the association being greatest in the group with the highest change in protein intake

($p<0.001$). The results were consistent when the study population was divided into subgroups based on physical activity and calorie intake.

Conclusions

The results of this study suggest that increasing protein intake may help to attenuate muscle loss in women over 40 years of age. The positive association between change of protein intake and change of muscle mass was found, and this association was greatest in the group with the highest change in protein intake. The study is novel in that it is a follow-up study limited to middle-aged women and uses the Appendicular Skeletal Muscle index (ASMi) as the primary outcome measure. The study also confirms the importance of physical activity in muscle formation and the positive effects of protein intake when accompanied by physical activity. However, further research is needed to fully understand the impact of changes in protein intake on muscle mass in middle-aged men.

Keyword : Muscle mass index, Protein intake, Middle-aged Korean women
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Chapter 1. Introduction

1.1. Study Background

In the population with sarcopenia (continuous muscle loss), appendicular skeletal muscle mass decreases, the basal metabolic rate decreases, and the surplus energy becomes fat, which leading to obesity or diabetes because it cannot be properly stored(1, 2). Diabetes and insulin resistance is not only a result of sarcopenia, but also a chronic inflammation is triggered by it(1). Moreover, chronic inflammation and insulin resistance are also known to increase the risk of dementia. A sharp decrease in muscle mass not only causes these chronic diseases, but also causes more severe musculoskeletal injuries due to falls(3-5). Thus, to reduce these risks (diabetes, dementia, and severe musculoskeletal injuries), maintaining muscle mass is important.

There are a number of factors related to decreased muscle mass(5). Even in healthy people, lose 1% of their muscle mass every year after middle age. External factors, including the diabetes and smoking, induced sarcopenia (1, 2). It is known that women with diabetes have a 2.92 times higher risk of accelerated muscle mass loss than women without diabetes. Smokers were 3.53 times more likely to lose muscle mass than nonsmokers(6).

1.2. Needs

Therefore, prevention of sarcopenia is an arising important health issue (4) as increasing life expectancy and decreasing activity in the aging population. In the healthy or unhealthy population, with aging, muscle mass and muscle strength, especially skeletal muscle mass and strength (hand grip), has generally decreased, while body fat mass, including abdominal and visceral fat mass, has increased, which is a general phenomenon of changes in body composition. , This changes in body composition causes a variety of health problems, including cardiovascular disease and dementia, beyond simple loss of activity.(7)

Women after middle age are more susceptible to sarcopenia than young- aged women or similar aged men. Because men have more muscle mass than women,(8) the effect on muscle loss is relatively less than that of women. When women lose muscle mass, they may face more challenges in life. In particular, it is known that the prevalence of sarcopenia in middle-aged women is high. Due to menopause, which causes rapid body changes, inflammatory cytokines due to a decrease in sex hormones increase, which promotes muscle catabolism and aggravates sarcopenia(9, 10). Furthermore, due to the lowered muscle strength, the exercise type is biased toward aerobic exercise rather than strength exercise, which further accelerates sarcopenia. At the same time, sarcopenic obesity and chronic diseases due to menopause are accompanied, leading to complications(9).

1.3. Previous Study

Proper exercise and protein intake are important for maintaining such muscle mass(4). To maintain muscle mass, not only aerobic exercise such as walking, running, and swimming, but also climbing stairs, dumbbells, and squats are required. Studying changes in protein intake and muscle mass in middle-aged women is an important factor in analyzing the causes and prevention of sarcopenia in middle-aged women.

1.4. Study Purpose

Though this background, there is still a lack of relevant research in Korea. The purpose of this study was to analyze the correlation between the change in protein intake and the change in muscle mass in middle-aged women, and to find out other factors that affect them.

Chapter 2. Method

2.1. Study Population

All data of middle-aged and elder women over 40 years in Korea were derived from the urban cohort in the Health Examinees (HEXA) study which was investigated as part of Korean Genome and Epidemiology Study (KoGES) in the Republic of Korea. The urban cohort has been used in food nutrition research, previously, due to nutritional information including a validated 103-item of food frequency questionnaire, as known as FFQ test (11, 12). The urban cohort in the HEXA study conducted the initial investigation on sociodemographic, anthropometric, and nutritional information during the baseline period (2008-2013). Sequentially, the follow-up investigation was conducted in during the follow-up period (2012-2016). In our study, we tracked the participants through the urban cohort in the HEXA study.

A total of 42,481 women aged over 40 years, who lived in Korean cities, have done the health examinations and surveys twice at both baseline and follow-up periods. Among them, 2,151 and 508 participants without key variables (sociodemographic data, medical history, health habits, and results of lab tests) and information about daily protein intake were excluded, respectively. In addition, 99 participants were excluded due to missing anthropometric data including height, weight, waist circumference (WC), and body mass index (BMI). Finally, to reduce the deviation and error caused by predicted muscle mass calculated by a predictive equation, 640 participants, each of the upper and lower 1% of predicted muscle mass, were additionally excluded. In conclusion, the final study population consisted of 42,094 participants as shown as Figure 1.

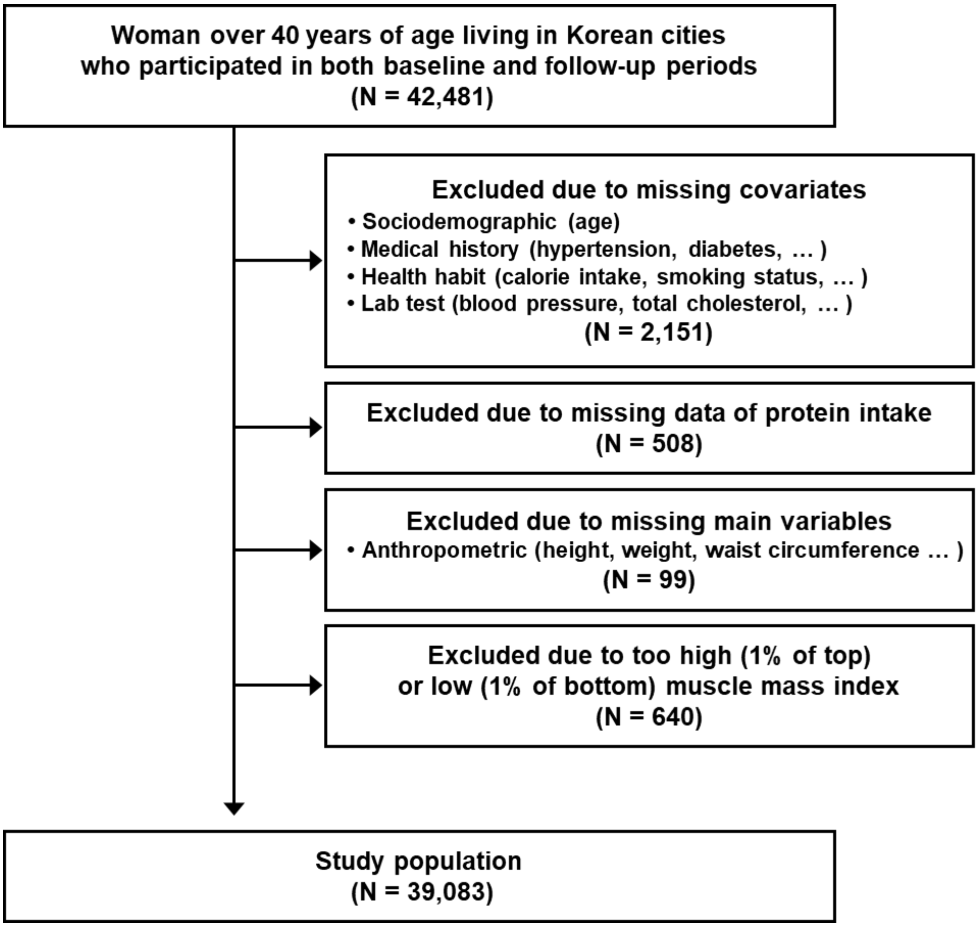

Figure 1. The Study population

2.2. Assessment of daily protein intake

Daily protein intake (g/days) in both baseline and follow-up periods was based on the result of the survey; “daily nutrient intake: protein (g/days)?”. The response to the survey was numerical and continuous value. Based on daily protein intake at two-time points, the difference between baseline and follow-up periods, changes in protein intake, was calculated. In the several studies, daily protein intake according to individual weight (g/days·kg) was usually used to compensate for the positive correlation between weight (or total calorie intake) and protein intake (13). In our study, however, crude protein intake was used as an exposure variable as in previous study (8). This is because un-adjusted protein intake (g) represents the levels of amino acid or protein that can be used in the body to grow muscle mass, independent on weight and total calorie intake. In addition, weight and muscle mass are highly related (Pearson correlation coefficient: r=0.839 at baseline periods and r=0.847 at follow-up periods). Thus, serious bias could exist when adjusted protein intake with weight (g/days·kg) is used. According to the 2020 Korean Nutrient Intake Standards, published by the Ministry of Health and Welfare of Korea, women over the age of 40 had a recommended intake of 50 g of protein: 30 to 49 years of age (50 g/days), 50 to 64 years of age (50 g/days), 64 to 74 years of age (50 g/days), and over 75 years of age (50 g/days). In our study, a total of 42,094 participants was classified into 5 groups according to the increase or decrease in protein intake based on ± 10 % (5 g) and 50% (25 g): the under <-25; -25≤, <-5; -5≤, <5; 5≤, <25; and ≥25 (g/days)

2.3. Key variables

Based on a prediction equation (14), predicted appendicular skeletal muscle mass index (ASMi) was derived. This proven equation has been used to calculate ASMi in previous studies (15). The prediction equation of ASMi was developed based on multiple linear regressions and Bland–Altman plots. The anthropometric prediction equation of ASMi consists of intercept and coefficients of multi-variables (sex, age, BMI, and WC). When assessed ASMi, this equation showed high predictive power, low bias, and moderate agreement. In our study, age, BMI, WC, and its correlation coefficient were used to calculate ASMi of women: ASMi =2.657-0.004·Age+0.190·BMI-0.008·WC. Information of 10,009 women, recruited through Korean National Health and Nutrition Examination Survey from 2008 to 2011, was divided into 7:3. Using information of approximately 7,000 women, a predictive equation was derived with multivariable linear regression. Then, using the remaining information of 3,003 women, the predicted value from the equation and the actual value were compared (method: Bland–Altman plot). The adjusted coefficient of determination, R^2, for muscle mass was 0.57, which is the 57% of the predicted outcome was very similar to the actual values. Thus, this predictive equation can be reasonably explained to 57% of case. In addition, only 5.59% of outcome was found to be outside the explainable range.

The covariates considered were sociodemographic data [age (continuous, years)], medical history [hypotension (categorical, yes or no), hyperlipidemia (categorical, yes or no), diabetes mellitus (categorical, yes or no), and osteoporosis (categorical, yes or no)], health habit [alcohol consumption (categorical, yes or no), smoking status (categorical, never, former, or current smoker), physical activity (categorical, low or high)], nutritional information: [daily calorie intake (continuous, kcal/days)], and others [follow-up duration (continuous, years) and body mass index (BMI; continuous, kg/m^2)]. The above covariates were derived from the results and answers investigated at baseline periods. For the stratified analysis, participants were classified according to changes in physical activity: physical activity Low-to-Low, Low-to-High, High-to-Low, and High-to-High. The survey and its responses on the exercise in the HEXA study were used to evaluate change in physical activity: "Do you exercise regularly enough to make your body sweat?" and the responses (Yes or No). In this study, response with 'Yes' was defined as high level of exercise, while it with 'No' as low level of exercise. Similarly, to consider the relation between calorie intake and protein intake, participants were additionally classified according to whether the calorie intake increased or decreased. Follow-up duration indicated the time interval between baseline and the follow-up periods. BMI was calculated by dividing body weight (kg) by the square of height (m^2).

2.4. Statistical analysis

Adjusted means and 95% confidence intervals (CI) of changes in ASMi were calculated using multiple liner regression analysis. Statistical significance was defined as *p*-value<0.05(*) or <0.001(#). *p*-value by chi-square test for categorical variables and analysis of variance (ANOVA) for continuous variables were used to determine the difference in general characteristics of the classified groups. After adjusting for several covariates, multiple linear regression was conducted to determine the adjusted mean (kg/m^2), and 95% confidence intervals (CI) of ΔASMi according to the change in protein intake. All data collection and analysis were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA)

2.4. Ethical approval and informed consent

There is no information on the participant's personal information in the results of our study. The Institutional Review Board (IRB) of the Seoul National University Hospital, which complies with the principles of the Declaration of Helsinki, approved this study (IRB no: 2208-020-1346). We conducted this research in compliance with the Declaration of Helsinki and ethical regulations.

Chapter 3. Results

During a mean follow-up of 4.99 years, protein intake was followed in 39,083 people in this study (Table 1). During the follow-up duration, 16% of participants reduced their protein intake by more than 25 g. Changes in protein intake during follow-up duration -25 g or more and less than -5 g were 12,703 people in the group, which was 32.5% of the total. This is the largest group of all groups. The group whose protein intake changed by more than -5 g and less than 5 g was 21.1% of the study population. 23% of the study population increased their protein intake by more than 5 g and less than 25 g. The group whose protein intake increased by 25 g, or more was the smallest group with 2,907 people, 7.4% of the total. BMI decreased with increasing changes in protein intake (p<0.001). Calorie intake also tends to decrease as the change in protein intake increases (p<0.001).

ASMi is shown as an indicator of the change in muscle mass according to change in protein intake (Table 2). The study population was divided into 5 groups according to change in protein intake (g/days). The change in ASMi of the group with change in protein intake less than -25 g/days was -0.03±0.23 kg/m^2. The change in ASMi of the group with a protein intake of -25 g/days or more to less than -5 g/days was -0.03±0.22 kg/m^2. The change in ASMi of the group with protein intake of -5 g/days or more to less than 5 g/days was -0.02±0.22 kg/m^2. The change in ASMi in the group with a protein intake of 5 g/days or more to less than 25 g/days was -0.01±0.22 kg/m^2. The change of ASMi in the group of 25 g/days or more was -0.01±0.23 kg/m^2.

Table 1. General characteristics of the study population.

	Range of change in protein intake [g/days]					*p*-value
	< -25	**-25 ≤ , < -5**	**-5 ≤ , < 5**	**5 ≤ , < 25**	**25 ≤**	
Study population, N (%)	6,260 (16.0)	12,703 (32.5)	8,232 (21.1)	8,981 (23.0)	2,907 (7.4)	
Protein intake [g/day], mean ± SD						
Baseline	84.82 ± 21.45	58.48 ± 15.09	48.49 ± 13.88	44.75 ± 14.67	44.84 ± 16.14	<0.001
Follow-up	43.91 ± 14.69	44.71 ± 14.44	48.39 ± 13.84	57.76 ± 15.40	84.01 ± 21.52	<0.001
Age [years], mean ± SD	52.26 ± 7.68	52.90 ± 7.76	53.24 ± 7.66	53.12 ± 7.61	52.91 ± 7.44	<0.001
Age [years], N (%)						<0.001
< 45	1,166 (18.6)	2,123 (16.7)	1,223 (14.9)	1,346 (15.0)	447 (15.4)	
45-59	3,880 (62.0)	7,789 (61.3)	5,125 (62.3)	5,617 (62.5)	1,848 ((63.6)	
≥ 60	1,214 (19.4)	2,791 (22.0)	1,884 (22.9)	2,018 (22.5)	612 (21.0)	
Follow-up duration [years], mean ± SD	5.25 ± 1.84	5.02 ± 1.75	4.90 ± 1.78	4.88 ± 1.83	4.93 ± 1.95	<0.001
Follow-up duration [years], N (%)						<0.001
1-3	1,726 (27.6)	4,088 (32.2)	2,886 (35.1)	3,245 (36.1)	1,052 (36.2)	
4-6	3,504 (56.0)	6,879 (54.1)	4,261 (51.8)	4,536 (50.5)	1,422 (48.9)	
7-9	902 (14.4)	1,572 (12.4)	966 (11.7)	1,068 (11.9)	380 (13.1)	
Over 10	128 (2.0)	167 (1.3)	119 (1.4)	132 (1.5)	53 (1.8)	
Medical history, N (%)						
Hypertension	1,025 (16.4)	2,302 (18.1)	1,502 (18.2)	1,648 (18.4)	502 (17.3)	0.011
Hyperlipidemia	657 (10.5)	1,393 (11.0)	935 (11.4)	965 (10.7)	297 (10.2)	0.345
Diabetes mellitus	319 (5.1)	703 (5.5)	453 (5.5)	418 (4.6)	138 (4.8)	0.024
Osteoporosis	412 (6.6)	951 (7.5)	666 (8.1)	701 (7.8)	199 (6.8)	0.005
BMI [kg/m^2], mean ± SD	23.60 ± 2.69	23.57 ± 2.73	23.48 ± 2.70	23.44 ± 2.67	23.39 ± 2.69	<0.001
BMI [kg/m^2], N (%)						0.021
< 18.5	113 (1.8)	202 (1.6)	154 (1.9)	177 (2.0)	54 (1.9)	
18.5-25.0	4,391 (70.1)	8,974 (70.6)	5,860 (71.2)	6,444 (71.8)	2,119 (72.9)	
≥ 25.0	1,756 (28.0)	3,527 (27.8)	2,218 (26.9)	2,360 (26.3)	734 (25.2)	
Calorie intake [kcal/day], mean ± SD	2,233 ± 507	1,745 ± 392	1530 ± 374	1,424 ± 400	1,405 ± 446	<0.001
Calorie intake [kcal/day], N (%)						<0.001
Low (< 1,500)	273 (4.4)	3,233 (25.4)	3,906 (47.4)	5,285 (58.8)	1,715 (59.0)	

Middle (1,500-2,000)	1,933 (30.9)	6,597 (51.9)	3,525 (42.8)	3,025 (33.7)	942 (32.4)	
High (≥ 2,000)	4,053 (64.8)	2,873 (22.6)	801 (9.7)	671 (7.5)	250 (8.6)	
Alcohol consumption, N (%)						0.004
No	4,370 (69.8)	9,120 (71.8)	5,982 (72.7)	6,472 (72.1)	2,090 (71.9)	
Yes	1,890 (30.2)	3,583 (28.2)	2,250 (27.3)	2,509 (27.9)	817 (28.1)	
Smoking status, N (%)						0.868
None	6,093 (97.3)	12,355 (97.3)	8,027 (97.5)	8,747 (97.4)	2,826 (97.2)	
Former	64 (1.0)	138 (1.1)	81 (1.0)	97 (1.1)	26 (0.9)	
Current	103 (1.6)	210 (1.6)	124 (1.5)	137 (1.5)	55 (1.9)	
Physical activity, N (%)						<0.001
Low	2,684 (42.9)	5,936 (46.7)	3,932 (47.8)	4,155 (46.3)	1,333 (45.8)	
High	3,575 (57.1)	6,767 (53.3)	4,300 (52.2)	4,826 (53.7)	1,574 (54.2)	
Anthropometry, mean ± SD						
Systolic blood pressure [mmHg]	120.52 ± 15.58	121.05 ± 16.14	121.33 ± 16.26	120.95 ± 15.77	119.66 ± 15.50	<0.001
Diastolic blood pressure [mmHg]	74.52 ± 9.86	74.55 ± 10.13	74.54 ± 10.23	74.51 ± 10.10	74.17 ± 9.75	0.455
Total cholesterol [mg/dL]	200.26 ± 36.05	199.48 ± 35.80	198.88 ± 35.06	199.64 ± 35.08	199.07 ± 35.60	0.201
Fasting glucose [mg/dL]	92.41 ± 17.41	92.47 ± 17.19	92.49 ± 17.29	92.40 ± 16.81	91.87 ± 15.24	0.516

adjusted means were calculated using multiple liner regression analysis after adjusting for age, follow-up duration, medical history, BMI, calorie intake, alcohol consumption, smoking status physical activity, and protein intake at baseline periods.
Abbreviation: number of population (N); standard derivation (SD); body mass index (BMI).

Table 2. Association between change in protein intake and muscle mass.

	Range of change in protein intake [g/days]					p_{trend}
	< -25	**-25 ≤ , < -5**	**-5 ≤ , < 5**	**5 ≤ , < 25**	**25 ≤**	
Study population, N	6,260	12,703	8,232	8,981	2,907	
ΔASMi, mean ± SD [kg/m^2]	-0.03 ± 0.23	-0.03 ± 0.22	-0.02 ± 0.22	-0.01 ± 0.22	-0.01 ± 0.23	
Linear regression						
adjusted mean (95 % CI)						
Model 1	-0.075 (-0.084, -0.066)	-0.064 (-0.071, -0.057)	-0.055 (-0.063, -0.047)	-0.047 (-0.055, -0.039)	-0.047 (-0.058, -0.037)	<0.001
p-value (Ref. **< -25**)	**Reference**	0.007**	<0.001#	<0.001#	<0.001#	
p-value (Ref. **-25≤, < -5**)	<0.001#	0.004**	**Reference**	0.017*	0.115	
Model 2	-0.062 (-0.075, -0.049)	-0.050 (-0.062, -0.039)	-0.042 (-0.054, -0.030)	-0.034 (-0.046, -0.023)	-0.036 (-0.049, -0.022)	<0.001
p-value (Ref. **< -25**)	**Reference**	0.002**	<0.001#	<0.001#	<0.001#	
p-value (Ref. **-25≤, < -5**)	<0.001#	0.008**	**Reference**	0.024*	0.186	
Model 3	-0.054 (-0.067, -0.041)	-0.042 (-0.054, -0.031)	-0.034 (-0.046, -0.022)	-0.026 (-0.039, -0.014)	-0.028 (-0.042, -0.014)	<0.001
p-value (Ref. **< -25**)	**Reference**	0.003**	<0.001#	<0.001#	<0.001**	
p-value (Ref. **-25≤, < -5**)	<0.001	0.009**	**Reference**	0.022*	0.199	

adjusted means were calculated using multiple liner regression analysis after adjusting for the following covariates:
Model 1: age, follow-up duration, medical history, and protein intake at baseline periods.
Model 2: Model 1 + BMI, calorie intake, alcohol consumption, smoking status, and physical activity.
Model 3. Model 2 + systolic and diastolic blood pressure, total cholesterol, and fasting glucose.
Abbreviation: number of population (N); standard derivation (SD); confidence interval (CI); change in appendicular skeletal muscle mass index (ASMi) during the periods (ΔASMi).
**p*<0.05, ***p*<0.01, and #*p*<0.001.

When each group classified according to change in protein intake is adjusted with the variable of Model 1, the change amount of ASMi in the group less than -25 g/days is -0.075 kg/m^2. The change in ASMi in the group with -25 g/days or more and less than -5 g/days was -0.064 kg/m^2 (p-value = 0.007 compared to the group less than -25 g/days), The change in ASMi in the group of -5 g/days or more and less than 5 g/days was -0.055 kg/m^2 (p-value <0.001). The change in ASMi in the group with 5 g/days or more and less than 25 g/days was -0.047 kg/m^2 (p-value <0.001). The change amount of ASMi in the group of 5 g/days or more was -0.047 kg/m^2 (p-value <0.001).

Similarly, when each group is adjusted using the variables of Model 2, the amount of change in ASMi in the group less than -25 g/days is -0.062 kg/m^2. the amount of change in ASMi in the group of -25 g/days or more and less than -5 g/days was -0.050 kg/m^2 (p-value = 0.002 based on the group less than -25 g/days), and the amount of change in ASMi in the group of -5 g/days or more and less than 5 g/days is -0.042 kg/m^2 (p-value <0.001 for groups less than -25 g/days). The amount of change in ASMi in the group with 5 g/days or more and less than 25 g/days was -0.034 kg/m^2 (p-value <0.001 for the group less than -25 g/days). The amount of change in ASMi in the group of 25 g/days or more was -0.036 kg/m^2 (p-value <0.001 based on the group less than -25 g/days).

In addition, when each group was adjusted using the variables of Model 3, the amount of change in ASMi in the group less than -25g/days was -0.054 kg/m^2. The amount of change in ASMi in the group of -25 g/days or more and less than -5 g/days was -0.042 kg/m^2 (p-value = 0.003 based on the group less than -25 g/days), and the amount of change in ASMi in the group of -5 g/days or more and less than 5 g/days is -0.034 kg/m^2 (p-value <0.001 for groups less than -25 g/days). The amount of change in ASMi in the group with 5 g/days or more and less than 25 g/days was -0.026 kg/m^2 (p-value <0.001 for the group less than -25 g/days). The amount of change in ASMi in the group of 5 g/days or more was -0.028 kg/m^2 (p-value <0.001 based on the group less than -25 g/days).

The effect of change in protein intake on ASMi is shown by dividing it according to the degree of physical activity (Table 3). Physical activity was divided into Low-to-Low (10,579 people), Low-to-High (7,462 people), High-to-Low (6,008 people), and High-to-High (15,034 people) groups depending on the baseline activity and the activity at the time of follow-up.

Table 3. Association between change in protein intake and muscle mass with change in physical activity.

	Range of change in protein intake [g/days]					p_{trend}
	< -25	**-25 ≤ , < -5**	**-5 ≤ , < 5**	**5 ≤ , < 25**	**25 ≤**	
			Physical activity: Low-to-Low			
Study population, N	1,543	3,498	2,324	2,475	739	
ΔASMi, mean ± SD [kg/m^2]	-0.02 ± 0.24	-0.02 ± 0.23	-0.02 ± 0.23	-0.00 ± 0.23	0.00 ± 0.26	
Linear regression						
adjusted mean (95 % CI)	-0.053 (-0.078, -0.029)	-0.034 (-0.056, -0.013)	-0.031 (-0.054, -0.009)	-0.012 (-0.035, 0.010)	-0.010 (-0.037, 0.016)	<0.001
p-value (Ref. **< -25**)	**Reference**	0.017*	0.016*	<0.001#	<0.001#	
p-value (Ref. **-25≤, < -5**)	0.016*	0.610	**Reference**	0.004**	0.030*	
			Physical activity: Low-to-High			
Study population, N	1,142	2,438	1,608	1,680	594	
ΔASMi, mean ± SD [kg/m^2]	-0.05 ± 0.25	-0.04 ± 0.22	-0.03 ± 0.22	-0.03 ± 0.22	-0.03 ± 0.22	
Linear regression						
adjusted mean (95 % CI)	-0.060 (-0.090, -0.031)	-0.050 (-0.076, -0.023)	-0.038 (-0.066, -0.011)	-0.032 (-0.060, -0.005)	-0.032 (-0.063, 0.000)	0.004
p-value (Ref. **< -25**)	**Reference**	0.243	0.033*	0.009**	0.028*	
p-value (Ref. **-25≤, < -5**)	0.033*	0.114	**Reference**	0.448	0.533	
			Physical activity: High-to-Low			
Study population, N	1,002	1,879	1,298	1,383	446	
ΔASMi, mean ± SD [kg/m^2]	-0.02 ± 0.23	-0.01 ± 0.23	-0.00 ± 0.24	0.00 ± 0.22	0.01 ± 0.21	
Linear regression						
adjusted mean (95 % CI)	-0.058 (-0.090, -0.026)	-0.045 (-0.074, -0.016)	-0.035 (-0.065, -0.004)	-0.028 (-0.058, 0.002)	-0.022 (-0.057, 0.012)	0.004
p-value (Ref. **< -25**)	**Reference**	0.213	0.044*	0.013*	0.015*	
p-value (Ref. **-25≤, < -5**)	0.044*	0.194	**Reference**	0.466	0.314	
			Physical activity: High-to-High			
Study population, N	2,573	4,888	3,002	3,443	1,128	
ΔASMi, mean ± SD [kg/m^2]	-0.04 ± 0.22	-0.04 ± 0.21	-0.03 ± 0.22	-0.02 ± 0.21	-0.03 ± 0.21	
Linear regression						
adjusted mean (95 % CI)	-0.076 (-0.096, -0.055)	-0.066 (-0.085, -0.047)	-0.057 (-0.076, -0.037)	-0.055 (-0.075, -0.036)	-0.062 (-0.084, -0.040)	0.015
p-value (Ref. **< -25**)	**Reference**	0.102	0.005**	0.003**	0.107	
p-value (Ref. **-25≤, < -5**)	0.005**	0.048*	**Reference**	0.829	0.460	

adjusted means were calculated using multiple liner regression analysis after adjusting for age, follow-up duration, medical history, BMI, calorie intake, alcohol consumption, smoking status physical activity, and protein intake at baseline periods.
Abbreviation: number of population (N); standard derivation (SD); confidence interval (CI); change in appendicular skeletal muscle mass index (ASMi) during the periods (ΔASMi).
*$p<0.05$, **$p<0.01$, and #$p<0.001$.
p for interaction = 0.188.

In the group with low-to-low physical activity, the amount of change in ASMi in the group with change in protein intake less than -25g/days was -0.02±0.24 kg/m^2, and the amount of change in ASMi in the group of -25 g/days or more to less than -5 g/days was -0.02±0.23 kg/m^2. The amount of change in ASMi in the group with a protein intake of -5g/days or more to less than 5g/days was -0.02±0.23 kg/m^2. The amount of change in ASMi in the group with a protein intake of 5 g/days or more to less than 25 g/days was -0.00±0.23 kg/m^2. The amount of change in ASMi in the group of 25 g/days or more was -0.00±0.26 kg/m^2. When linear regression was performed for each group, the amount of change in ASMi in the group less than -25g/days was -0.053 kg/m^2. The amount of change in ASMi in the group with -25 g/days or more and less than -5 g/days was -0.034 kg/m^2 (p-value = 0.017 based on the group less than -25 g/days) and the change in ASMi in the group with -5 g/days or more and less than 5 g/days is -0.031 kg/m^2 (p-value = 0.016 based on the group less than -25 g/days). The amount of change in ASMi in the group with 5 g/days or more and less than 25 g/days is -0.012 kg/m^2 (p-value <0.001 for the group less than -25 g/days). The amount of change in ASMi in the group of 25 g/days or more was -0.010 kg/m^2 (p-value <0.001 based on the group below -25g/days).

In the group with low-to-high physical activity, the amount of change in ASMi in the group with change in protein intake less than -25g/days was -0.05±0.25 kg/m^2, and the amount of change in ASMi in the group of -25 g/days or more to less than -5 g/days was -0.04±0.22 kg/m^2. The change in ASMi in the group with -5 g/days or more and less than 5 g/days is -0.03±0.22 kg/m^2. The amount of change in ASMi in the group with 5 g/days or more and less than 25 g/days is -0.03±0.22 kg/m^2. The amount of change in ASMi in the group of 25 g/days or more is -0.03±0.22 kg/m^2. When linear regression was performed for each group, the amount of change in ASMi in the group less than -25g/days was -0.060 kg/m^2. The amount of change in ASMi in the group with -25 g/days or more and less than -5 g/days was -0.050 kg/m^2 (p-value = 0.243 based on the group less than -25 g/days), and the change in ASMi in the group with -5 g/days or more and less than 5 g/days is -0.038 kg/m^2 (p-value

= 0.033 based on the group less than -25 g/days). The amount of change in ASMi in the group with 5 g/days or more and less than 25 g/days is -0.032 kg/m^2 (p-value = 0.009 based on the group less than -25 g/days). The amount of change in ASMi in the group of 25 g/days or more is -0.032 kg/m^2 (p-value = 0.028 based on the group less than -25 g/days).

In the group with high-to-low physical activity, the amount of change in ASMi in the group with change in protein intake less than -25g/days is -0.02±0.23 kg/m^2, and the amount of change in ASMi in the group of -25 g/days or more to less than -5 g/days was -0.01±0.23 kg/m^2. The change in ASMi in the group with -5 g/days or more and less than 5 g/days is -0.00±0.24 kg/m^2. The amount of change in ASMi in the group with 5 g/days or more and less than 25 g/days is 0.00±0.22 kg/m^2. The amount of change in ASMi in the group of 25 g/days or more is 0.01±0.21 kg/m^2. When linear regression was performed for each group, the amount of change in ASMi in the group less than -25g/days is -0.058 kg/m^2. The amount of change in ASMi in the group with -25 g/days or more and less than -5 g/days is -0.045 kg/m^2 (p-value = 0.213 based on the group less than -25 g/days), and the change in ASMi in the group with -5 g/days or more and less than 5 g/days is -0.035 kg/m^2 (p-value = 0.044 based on the group less than -25 g/days). The amount of change in ASMi in the group with 5 g/days or more and less than 25 g/days is -0.028 kg/m^2 (p-value = 0.013 based on the group less than -25 g/days). The amount of change in ASMi in the group of 25 g/days or more is -0.022 kg/m^2 (p-value = 0.015 based on the group less than -25 g/days).

In the group with high-to-high physical activity, the amount of change in ASMi in the group with change in protein intake less than -25g/days is -0.04±0.22 kg/m^2, and the amount of change in ASMi in the group of -25 g/days or more to less than -5 g/days is -0.04±0.21 kg/m^2. The change in ASMi in the group with -5 g/days or more and less than 5 g/days is -0.03±0.22 kg/m^2. The amount of change in ASMi in the group with 5 g/days or more and less than 25 g/days is 0.02±0.21 kg/m^2. The amount of change in ASMi in the group of 25 g/days or more is 0.03±0.21 kg/m^2.

When linear regression was performed for each group, the amount of change in ASMi in the group less than -25g/days is -0.076 kg/m^2. The amount of change in ASMi in the group with -25 g/days or more and less than -5 g/days is -0.066 kg/m^2 (p-value = 0.102 based on the group less than -25 g/days) and the change in ASMi in the group with -5 g/days or more and less than 5 g/days is -0.057 kg/m^2 (p-value = 0.005 based on the group less than -25 g/days). The amount of change in ASMi in the group with 5 g/days or more and less than 25 g/days is -0.055 kg/m^2 (p-value = 0.003 based on the group less than -25 g/days). The amount of change in ASMi in the group of 25 g/days or more is -0.062 kg/m^2 (p-value = 0.0107 based on the group less than -25 g/days).

The effect of change in protein intake on ASMi is shown by dividing it according to the increase and decrease of calorie intake (Table 4). Calorie intake was divided into Calorie intake decrease (23,693 persons) and Calorie intake increase (15,390 persons) groups according to the baseline intake and intake at follow-up.

Table 4. Association between change in protein intake and muscle mass with change in calorie intake.

	Range of change in protein intake [g/days]					p_{trend}
	< -25	**-25 ≤ , < -5**	**-5 ≤ , < 5**	**5 ≤ , < 25**	**25 ≤**	
			Calorie intake decreases			
Study population, N	6,193	11,270	4,585	1,571	74	
ΔCalorie, mean ± SD [kcal]	-904 ± 444	-428 ± 263	-227 ± 182	-173 ± 158	- 145 ± 162	
ΔASMi, mean ± SD [kg/m^2]	-0.03 ± 0.23	-0.03 ± 0.23	-0.02 ± 0.23	-0.02 ± 0.22	-0.02 ± 0.22	
Linear regression						
adjusted mean (95 % CI)	-0.064 (-0.079, -0.049)	-0.056 (-0.070, -0.041)	-0.049 (-0.064, -0.034)	-0.050 (-0.068, -0.032)	-0.054 (-0.106, -0.002)	0.002
p-value (Ref. **< -25**)	**Reference**	0.027*	0.001**	0.027*	0.687	
p-value (Ref. **-5 ≤ , < 5**)	0.001**	0.079	**Reference**	0.875	0.848	
			Calorie intake increases			
Study population, N	67	1,433	3,647	7,410	2,833	
ΔCalorie, mean ± SD [kcal]	162 ± 184	161 ± 151	190 ± 166	356 ± 242	770 ± 406	
ΔASMi, mean ± SD [kg/m^2]	-0.01 ± 0.22	-0.02 ± 0.23	-0.02 ± 0.22	-0.01 ± 0.22	-0.01 ± 0.23	
Linear regression						
adjusted mean (95 % CI)	-0.006 (-0.061, 0.049)	-0.027 (-0.047, -0.006)	-0.032 (-0.050, -0.013)	-0.026 (-0.044, -0.008)	-0.029 (-0.048, -0.010)	0.256
p-value (Ref. **< -25**)	**Reference**	0.440	0.338	0.451	0.398	
p-value (Ref. **-25≤, < -5**)	0.338	0.490	**Reference**	0.209	0.589	

adjusted means were calculated using multiple liner regression analysis after adjusting for age, follow-up duration, medical history, BMI, calorie intake, alcohol consumption, smoking status physical activity, and protein intake at baseline periods.

Abbreviation: number of population (N); standard derivation (SD); confidence interval (CI); change in appendicular skeletal muscle mass index (ASMi) during the periods (ΔASMi); change in calorie intake during the periods (ΔCalorie).

*p<0.05, **p<0.01, and #p<0.001.

p for interaction = 0.430.

Within the group where calorie intake decreases, the amount of change in ASMi in the group with change in protein intake less than -25g/days is -0.03±0.23 kg/m^2, and the amount of change in ASMi in the group of -25 g/days or more to less than -5 g/days is -0.03±0.23 kg/m^2. The change in ASMi in the group with -5 g/days or more and less than 5 g/days is -0.02±0.23 kg/m^2. The amount of change in ASMi in the group with 5 g/days or more and less than 25 g/days is -0.02±0.22 kg/m^2. The amount of change in ASMi in the group of 25 g/days or more is -0.002±0.22 kg/m^2. When linear regression was performed for each group, the amount of change in ASMi in the group less than -25g/days is -0.064 kg/m^2. The amount of change in ASMi in the group with -25 g/days or more and less than -5 g/days is -0.056 kg/m^2 (p-value = 0.027 based on the group less than -25 g/days), and the change in ASMi in the group with -5 g/days or more and less than 5 g/days is -0.049 kg/m^2 (p-value = 0.001 based on the group less than -25 g/days). The amount of change in ASMi in the group with 5 g/days or more and less than 25 g/days is -0.050 kg/m^2 (p-value = 0.027 based on the group less than -25 g/days). The amount of change in ASMi in the group of 25 g/days or more is -0.054 kg/m^2 (p-value = 0.687 based on the group less than -25 g/days).

Within the group where calorie intake increases, the amount of change in ASMi in the group with change in protein intake less than -25g/days is -0.01±0.22 kg/m^2, and the amount of change in ASMi in the group of -25 g/days or more to less than -5 g/days is -0.02±0.23 kg/m^2. The change in ASMi in the group with -5 g/days or more and less than 5 g/days is -0.02±0.22 kg/m^2. The amount of change in ASMi in the group with 5 g/days or more and less than 25 g/days is -0.01±0.22 kg/m^2. The amount of change in ASMi in the group of 25 g/days or more is -0.001±0.23 kg/m^2. When linear regression was performed for each group, the amount of change in ASMi in the group less than -25g/days is -0.006 kg/m^2. The amount of change in ASMi in the group with -25 g/days or more and less than -5 g/days is -0.027 kg/m^2 (p-value = 0.440 based on the group less than -25 g/days), and the change in ASMi in the group with -5 g/days or more and less than 5 g/days is -0.032 kg/m^2 (p-value

= 0.338 based on the group less than -25 g/days). The amount of change in ASMi in the group with 5 g/days or more and less than 25 g/days -0.026 kg/m^2 (p-value = 0.451 based on the group less than -25 g/days). The amount of change in ASMi in the group of 25 g/days or more is -0.029 kg/m^2 (p-value = 0.398 based on the group less than -25 g/days).

Changes in the appendicular skeletal muscle index according to protein intake changes in several study population groups were visualized using a restricted cubic spline curve (RCS curve) (Figure 2). In the RCS curve of the overall study population, change in protein intake 0~20 g/day showed the largest change of adjustment mean of ASMi (Figure 2a). Additionally, to confirm whether physical activity has an effect on association of change in protein intake with ΔASMi, we confirm the RCS curve by dividing the study population into 4 groups according to the change in physical activity at baseline and follow-up. In the physical activity low-to-low group, the largest adjustment mean of ASMi change was shown at 0~20 g/day of change in protein intake (Figure 2b). In the physical activity low-to-high group, the largest adjustment mean of ASMi change was shown at -20~0 g/day of change in protein intake (Figure 2c). In the physical activity high-to-low, the largest adjustment mean of ASMi change was shown at 0~20 g/day of change in protein intake (Figure 2d). In the physical activity low-to-low, the largest adjustment mean of ASMi change was shown at -20~0 g/day of change in protein intake (Figure 2e). In addition, separate from change in protein intake, to determine whether change in calorie intake has an effect on the association of change in protein intake with ΔASMi, we confirm the RCS curve by dividing the study population into 2 groups according to the increase or decrease of calorie intake during the follow-up duration. In the calorie intake decreases group, the largest adjustment mean of ASMi change was shown at -20~0 g/day of change in protein intake (Figure 2f). In the calorie intake decreases group, the study population with increased protein intake is less than the study population with decreased protein intake. On the other hand, in the calorie intake increases group, the largest adjustment mean of ASMi change was shown at 0~20 g/day of change in protein intake (Figure 2g). In the calorie intake increases group, the study population with increased protein intake is more than the study population with decreased protein intake.

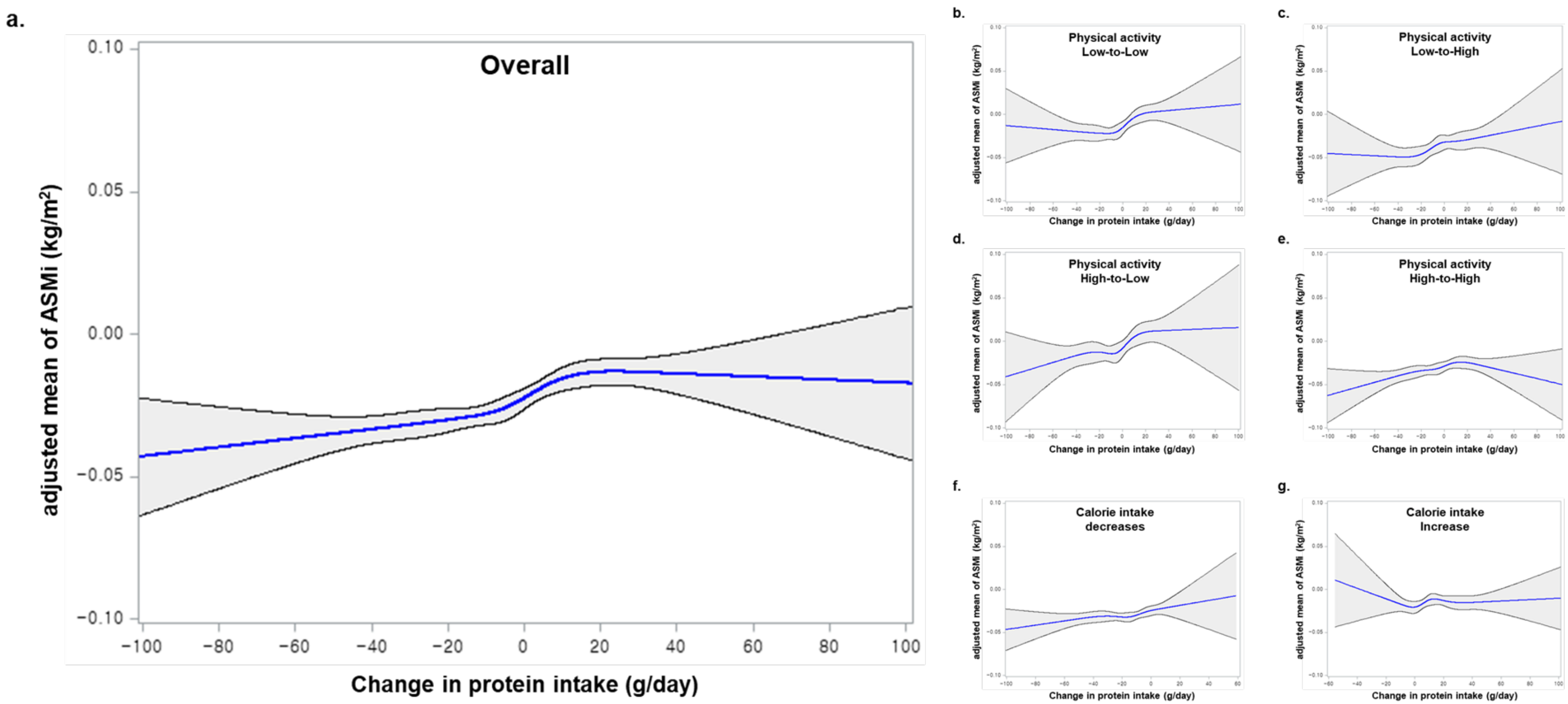

Figure 2. Restricted cubic spline curve (RCS). a). Association of change in protein intake with change in predicted appendicular skeletal muscle mass index (ΔASMi). Adjusted mean of ΔASMi according to change in protein intake stratified by change in physical activity: b). Low-to-Low, c). Low-to-High, d). High-to-Low, and e). High-to-High. Adjusted mean of ΔASMi according to change in protein intake stratified by change in calorie intake: f). calorie intake decreases and g). calorie intake increases. Blue solid lines indicate adjusted mean and the grey shaded regions show 95% confidence intervals from RCS. RCS was constructed with five knots placed at the 5th, 25th, 50th, 75th, and 95th percentiles of change in protein intake. Adjusted means were calculated using multiple liner regression analysis after adjusting for age, follow-up duration, medical history, BMI, calorie intake, alcohol consumption, smoking status, physical activity, and protein intake at baseline periods.

We did stratified analysis with specific variables of change in ASMi according to change in protein intake (Table 5). In the case of stratified analysis of Age for each group of change in protein intake, the change in ASMi according to the increase in change in protein intake tends to increase statistically significantly for the groups 45 or more and 59 or less years old and above 60 years old. In the group under 45 years of age, increasing protein intake increased ASMi. In the group 45 or more and 59 or less years old, when protein intake was increased, the decrease in ASMi was attenuated. Also, for those above 60 years old, increasing protein intake attenuates the decrease of ASMi. However, even in groups with the same change in protein intake, the decrease in ASMi was greater with age. In the case of stratified analysis of BMI, the change in ASMi according to the increase in change in protein intake tends to increase statistically significant for the group or less 25 kg/m^2 and above 25 kg/m^2. As a result of stratified analysis of hypertension, the change in ASMi according to the increase in change in protein intake tends to increase statistically significantly in both cases with and without hypertension. In the case of stratified analysis of osteoporosis, in the absence of osteoporosis, the change in ASMi according to the increase in change in protein intake tends to increase statistically significantly, but, in the presence of osteoporosis, the change in ASMi according to the increase in change in protein intake does not show a statistically significant tendency. We stratified analysis with follow-up duration, for the groups with follow-up duration from 1 to 3 years and from 4 to 6 years, the change in ASMi according to the increase in change in protein intake showed a tendency to significantly increase, In the group with an up duration of 7 to 12 years, the change in ASMi according to the increase in change in protein intake did not show a significant tendency. A stratified analysis of calorie intake shows that the change in ASMi significantly increases in the Low (<1,500 kcal/day) and Middle (1,500-2,000 kcal/day) groups as the change in protein intake increases. However, in the High (≥2,000 kcal/day) group, there was no statistically significant tendency for the change in ASMi according to the increase in change in protein intake.

Table 5. Stratified analysis according to age, BMI, underlying disease, follow-up duration, and calorie intake.

	Range of change in protein intake [g/days]					$p_{inter-action}$
	< -25	-25 ≤ , < -5	-5 ≤ , < 5	5 ≤ , < 25	25 ≤	
Age [years]						0.533
< 45	0.024 (-0.028, 0.076)	0.013 (-0.038, 0.064)	0.032 (-0.020, 0.085)	0.041 (-0.011, 0.093)	0.048 (-0.007, 0.102)	
45-59	-0.062 (-0.078, -0.047)	-0.055 (-0.069, -0.04)	-0.052* (-0.067, -0.036)	-0.044# (-0.06, -0.029)	-0.043* (-0.06, -0.026)	
≥ 60	-0.079 (-0.109, -0.05)	-0.063 (-0.091, -0.036)	-0.056* (-0.084, -0.028)	-0.048* (-0.076, -0.020)	-0.061 (-0.093, -0.029)	
Menopause						0.672
No	-0.073 (-0.098, -0.048)	-0.078 (-0.102, -0.055)	-0.063 (-0.088, -0.039)	-0.056* (-0.081, -0.032)	-0.059 (-0.086, -0.033)	
Yes	-0.066 (-0.082, -0.051)	-0.050# (-0.064, -0.036)	-0.049# (-0.064, -0.034)	-0.041# (-0.056, -0.026)	-0.040# (-0.056, -0.026)	
BMI [kg/m^2]						<0.001
< 25.0	-0.032 (-0.046, -0.018)	-0.031 (-0.044, -0.018)	-0.023* (-0.037, -0.01)	-0.020* (-0.033, -0.006)	-0.018* (-0.033, -0.002)	
≥ 25.0	-0.119 (-0.145, -0.093)	-0.099* (-0.123, -0.076)	-0.096* (-0.120, -0.071)	-0.078# (-0.103, -0.053)	-0.088* (-0.117, -0.059)	
Hypertension						0.873
No	-0.054 (-0.068, -0.040)	-0.051 (-0.064, -0.038)	-0.044* (-0.058, -0.031)	-0.039* (-0.053, -0.026)	-0.033# (-0.049, -0.018)	
Yes	-0.091 (-0.121, -0.061)	-0.070* (-0.098, -0.043)	-0.064* (-0.092, -0.036)	-0.047# (-0.075, -0.018)	-0.080 (-0.113, -0.047)	
Osteoporosis						0.294
No	-0.047 (-0.059, -0.035)	-0.04 (-0.052, -0.029)	-0.033* (-0.045, -0.021)	-0.027# (-0.039, -0.015)	-0.027# (-0.041, -0.014)	
Yes	-0.069 (-0.113, -0.025)	-0.07 (-0.109, -0.031)	-0.073 (-0.112, -0.033)	-0.051 (-0.092, -0.01)	-0.056 (-0.105, -0.008)	
Follow-up duration [years]						<0.001
1-3	-0.057 (-0.078, -0.036)	-0.042* (-0.060, -0.023)	-0.036* (-0.055, -0.017)	-0.025# (-0.044, -0.005)	-0.030* (-0.052, -0.009)	
4-6	-0.062 (-0.078, -0.046)	-0.057 (-0.072, -0.042)	-0.052 (-0.068, -0.036)	-0.043# (-0.059, -0.027)	-0.040* (-0.058, -0.021)	
7-12	-0.041 (-0.081, -0.002)	-0.043 (-0.08, -0.005)	-0.026 (-0.065, 0.012)	-0.036 (-0.075, 0.002)	-0.037 (-0.080, 0.005)	
Calorie intake [kcal/day]						0.091
Low (< 1,500)	-0.066 (-0.098, -0.035)	-0.036* (-0.055, -0.018)	-0.031* (-0.049, -0.012)	-0.020# (-0.038, -0.003)	-0.022* (-0.042, -0.002)	
Middle (1,500-2,000)	-0.065 (-0.085, -0.045)	-0.061 (-0.079, -0.043)	-0.057 (-0.076, -0.038)	-0.052* (-0.071, -0.032)	-0.049 (-0.072, -0.027)	
High (≥ 2,000)	-0.063 (-0.088, -0.038)	-0.057 (-0.082, -0.032)	-0.040* (-0.069, -0.012)	-0.045* (-0.074, -0.016)	-0.056 (-0.092, -0.020)	

adjusted means were calculated using multiple liner regression analysis after adjusting for age, follow-up duration, medical history, BMI, calorie intake, alcohol consumption, smoking status, physical activity, and protein intake at baseline periods.
Abbreviation: body mass index (BMI); change in appendicular skeletal muscle mass index (ASMi) during the periods (ΔASMi)
**p*<0.05, ***p*<0.01, and #*p*<0.001 compared to the < -25 group.

Chapter 4. Discussion

In this population study, the effect of changes in protein intake on muscle loss in women over 40 years of age was investigated in depth. There were positive associations between change of protein intake and change of muscle mass. For most people, muscle mass gradually decreases by approximately 1% per year from middle age(9). Thus, our results implied that the loss of muscle mass may be attenuated with increasing protein intake. We also demonstrated the effects of physical activity and the change in protein intake on muscle formation can be found. To our knowledge, this is the first study to investigate the association between changes in protein intake and changes in muscle mass in Korean women over 40 years of age during the baseline and follow-up periods.

There were few previous studies on the association between change of protein and change of muscle mass in middle-aged women. But the association between dietary protein and muscle strength, muscle mass or lean mass is well conducted. One study was not associated with developing low muscle strength in middle-aged and older adults(3). However, there were several studies that support our results. Other studies show that higher dietary intakes of total, animal, and plant protein, regardless of the ratio of animal-to-plant protein, are associated with greater skeletal muscle mass(5). It reported that The SMI increased significantly across quintiles of relative dietary intake of total, animal, and plant protein (all P trends<0.001). Other Previous Studies have shown that there is a significant positive association between protein intake and the effects of muscle strength and muscle mass(5). Also, there were results that men and women with higher protein intakes had a higher lean mass after 12 years of follow-up(7). Consistent with the results of previous studies, we found positive association between protein intake and muscle mass, after adjusting for covariates. Although further research is needed to fully understand the impact of change in protein intake on change in muscle mass, one possible mechanism is the muscle protein synthesis is stimulated by amino acids(16). Physiologically, with age, anabolic resistance that is resistance to muscle maintenance and accretion may develop(17), thereby, a higher level of protein intake is likely to attenuate the loss of muscle because the synthesis is promoted by common pathway of action through activation of mTOR(18). Another mechanism that can support our result is that increasing circulating levels of insulin-like growth factor 1(IGF-1). It is known that the IGF-1 is also activating muscle protein synthesis by changing in muscle histology(19).

However, compared to young-aged women and men, among middle-aged women, the prevalence of sarcopenia is high not only because they undergo rapid physical changes due to menopause but also because the severity of injuries due to sarcopenia is more serious due to accompanying osteoporosis. Thus, middle-aged women are more susceptible to sarcopenia. In this respect, this study is novel because it is a follow-up study limited to middle-aged women.

Other studies used grip strength(7, 10), muscle mass(12), ASM(9), ASMi(12), etc. However, there has been a worrying about using of hand grip strength as the measure of evaluation in muscle strength(20). We calculated the Appendicular Skeletal Muscle index (ASMi) to confirm the change in muscle formation.

According to previous papers, it is known that physical activity is helpful for muscle formation, and protein intake is also known to improve muscle formation when physical activity is accompanied(21). According to the results of our study, it was confirmed that despite having less physical activity, muscle loss can be minimized if protein intake is increased. In the case of the group with high-to-high physical activity, the muscle mass decreased significantly in the group with reduced protein intake, based on the group with a change in protein intake (-5 g/days or more and less than 5 g/days). This implies that high physical activity with insufficient protein intake leads to a decrease in muscle mass. These results consistent with the previous study that protein intake is necessary to increase muscle mass through physical activity. (22) Other studies have shown that different types and intensities of physical activity have different effects on muscle synthesis and breakdown. (23) However, the data used in our study was insufficient data on different types and intensities of exercise. Therefore, we hope that the association between muscle mass and amount of exercise can be studied using a quantitative exercise amount calculation method from an appropriate database.

In this experiment, the average daily intake was used to evaluate the protein intake. In the preceding papers, the average daily intake (unit: g/days) was used to define nutrient intake, (9, 24) or the average daily intake per body weight (unit: g/kg·days) was used. (3, 5, 12) The use of average daily intake per body weight is used because there are different effects on people with different weights despite consuming the same nutrients. However, the average daily intake per body weight is not appropriate for follow-up studies with changes in body weight in the same sample. In this study, if protein intake is defined as the average daily intake per body weight, then protein intake is more dependent on changes in body weight than on changes in actual protein intake.

The strengths of this study include the following. First, rather than simply observing the protein intake at the time of measurement, the change in protein intake was followed up and observed. In fact, the change in protein intake in middle age is significant as demonstrated by 78.9% of the 39,083 experimental population in which protein intake had been increased or decreased by 5 g or more. Second, the change in muscle mass caused by the change in protein intake and the change in the amount of exercise was separately observed. In general, it is known that muscle mass is correlated with the change in the amount of exercise(25). And other previous studies also reported that the importance of exercise in middle-aged women to improve loss of muscle and their health(26). However, it’s difficult for middle-aged and older women to do more exercise. We showed from Figures 2b and 2d that increasing the protein intake when the amount of exercise is low or low can help alleviate the decrease in muscle mass when the physical activity is low or low. This means that increasing the protein intake of middle-aged women who are difficult to do exercise can be helpful for preventing sarcopenia.

The limitations of this study include the following. First, the effect of calorie intake could not be excluded. In the study population, protein intake and calorie intake showed a strong positive correlation (Baseline r=0.867, Follow-up r=0.860). Therefore, it cannot be assumed that calorie has no role in this phenomenon. However, based on the analysis of Table 4, it was confirmed that there is a constant trend whether the calorie is increased or decreased. Second, instead of having a continuous follow-up during the follow-up duration, two separate measurements were collected, one from baseline and another from follow-up. Daily protein intake can fluctuate greatly depending on the day. Thus, it is difficult to conclude that the intake on a specific day represents the intake for that period. Lastly, in evaluating the amount of exercise, it was not possible to distinguish between strength training, which is known to contribute to muscle formation, and exercise that has relatively no effect on muscle formation. Because cardiovascular exercise reduces body fat and lowers body weight, the ASMi calculated by the prediction equation may be relatively low.

In conclusion, in Korean middle-aged women, there was a positive association protein intake and muscle mass. The group with a higher change in protein intake has a higher change in muscle mass. Thus, it can be recommended to increase protein intake to preserve the muscle mass of middle-aged women. This help attenuate risks of sarcopenia especially middle-aged women in low physical activity individuals. Further study is required to confirm that the change in protein intake and change in muscle mass also have a positive association in middle-aged men.

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Abstract
요약(국문 초록)

목적

중년 이후의 여성은 다른 연령대나 남성들보다 근감소증에 취약하다. 특히, 근감소증은 중년 여성에 있어 염증성 사이토카인과 성호르몬의 불균형으로 인해 널리 퍼져있으며, 폐경기동안과 그 이후 근육 이화작용이 촉진된다. 중년 여성의 단백질 섭취와 근육량의 연관성에 대한 연구는 근감소증의 원인을 분석하고 예방하는데 중요하다. 본 연구의 목적은 중년 여성의 단백질 섭취량의 변화와 근육량의 변화 간의 상관관계를 분석하고, 근육 손실을 완화하기 위한 다른 요인을 조사하기 위함이다.

연구 방법

한국의 중년 및 노년 여성(40세 이상)의 모든 데이터는 건강검진자 연구의 도시코호트를 활용하였다. 본 연구에서는, 총 42,094명의 참가자를 단백질 섭취량의 증가 또는 감소에 따라 5개의 그룹: <−25; −25≤, <−5; −5≤, <5; 5≤, <25; and ≥25 (g/days)으로 분류하였다. 예측 방정식을 기반으로, 예측 사지 골격근 질량 지수(ASMi)를 계산하였다. ASMi의 변화에 대한 보정 평균과 95%의 신뢰구간(CI)은 다중선형회귀분석을 사용하여 계산하였다.

결과

추적 기간 동안, 참가자 중 16%는 단백질 섭취량이 25g 이상 감소하였고, 단백질 섭취량이 −5g 이상 5g 이하로 변화한 그룹은 21.1% 였다. 23%는 5g 이상 25g 이하로 단백질 섭취량이 변화한 그룹이다. 단백질 섭취량이 25g 이상 증가한 그룹은 전체 인구의 7.4% 인 2,907명이었고 가장 작은 비율을 보였다. BMI는 단백질 섭취량 변화가 증가할수록 감소하였다($p<0.001$). 칼로리 섭취량도 단백질 섭취량 변화가 증가할수록 감소하는 경향을 보였다($p<0.001$). 연구 대상 인구를 단백질 섭취량 변화에 따라 5개 그룹으로 나누어 각 그룹의 ASMi 변화를 조사하였다. 3가지 모델로 가능한 변수를 조정한 후에도, 단백질 섭취량 변화와 ASMi

변화 간에는 음의 상관관계가 있었으며, 이 연관성의 크기는 단백질 섭취량 변화가 가장 큰 그룹에서 가장 큰 것으로 보인다($p<0.001$). 운동량과 칼로리 섭취량에 따라 연구 대상 인구를 구분하여 조사한 결과도 일치하는 상관관계를 보였다.

결론

본 연구 결과는 40세 이상의 여성에서 단백질 섭취량의 증가가 근육 손실을 완화시키는데 도움이 될 수 있음을 제시한다. 단백질 섭취량의 변화와 근육 형성의 변화 사이에 양의 상관관계를 보였고, 이 상관 관계는 단백질 섭취량의 변화가 가장 큰 그룹에서 가장 크게 나타났다.

본 연구는 기존 연구와 다르게 중년 여성만을 대상으로 하는 추적관찰연구이고, 일차평가지표로써 사지 골격근 질량 지수(ASMi)를 사용한 점에 있어 신규성이 있다.

또한, 본 연구는 신체활동이 근육 형성에 중요하다는 것을 확인하였고, 단백질 섭취가 근육 형성에 미치는 효과는 신체활동을 동반했을 때 긍정적인 효과가 있다는 것을 입증하였다. 그러나, 중년 남성에 있어 단백질 섭취의 변화와 근육 형성과의 관계에 대해서는 추가 연구가 필요하다.

주요어: 근육량 지수, 단백질 섭취량, 중년 여성

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