Impact of dietary protein intake and obesity on lean mass in middle-aged individuals after a 12-year follow-up: The Korean Genome and Epidemiology Study (KoGES)

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Abstract

This study investigated the association between protein intake and lean mass according to obesity status over a 12-year period. Data on 4,412 participants aged 40–69 years were obtained from the Korean Genome and Epidemiology Study. The usual dietary protein intake of these participants was assessed at baseline using a semi-quantitative food frequency questionnaire. Body composition was measured using a bioelectrical impedance analysis at baseline and after a 12-year follow-up. Linear mixed effects models were used to examine the associations between lean mass after a 12-year follow-up and protein intake at baseline. After adjusting for covariates and lean mass at baseline, comparisons between the highest and lowest tertiles revealed that dietary protein intake was positively associated with lean mass in both men (β=0.79, p=0.001) and women (β=0.28, p=0.082) after the 12-year period; however, those differences were attenuated after additional adjustment for fat mass at baseline and were stronger in the normal-weight group (men, β=0.85, p=0.002; women, β=0.97, p<0.001) but were not detected in the obese group. In the obese group, age (men, β=4.08, p<0.001; women, β=2.61, p<0.001) and regular physical activity (men, β=0.88, p=0.054; women, β=0.76, p<0.001) were significantly associated with lean mass after 12 years of follow-up. The results of this study showed that protein intake may contribute to the prevention of ageing-related lean mass loss; however, the impact of this intake may vary depending on obesity status. Therefore, the maintenance of a healthy body weight during ageing through enhanced protein intake is likely to confer health benefits.
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1. Introduction

The number of individuals over 60 years of age worldwide is expected to increase from 900 million to 2 billion between 2015 and 2050 \(^1\), with a subsequent increase in the number of people at risk of deteriorating strength and mobility related to muscle loss. Recent estimates indicate that the prevalence of sarcopenia is 5%–13% in individuals 60–70 years of age and 11%–50% in those 80 years of age and older \(^2\). Sarcopenia is a syndrome characterized by the progressive and generalized loss of skeletal muscle mass and strength and is associated with an increased risk of falls and fractures, reduced cardiopulmonary function, metabolic syndrome, and insulin resistance; accordingly, this condition eventually results in disability, hospitalization, and death among older individuals \(^3\). Although there are few estimates of the financial burden of sarcopenia in older adults, the healthcare costs of this condition are likely to be high \(^4\). One prospective study estimated that sarcopenia would increase hospitalization costs by 58.5% and 34% for patients <65 and ≥65 years of age, respectively \(^5\).

Ageing is probably the most important factor contributing to the loss of muscle mass, although this decline can also be accelerated by modifiable lifestyle factors such as physical inactivity, smoking, alcohol consumption, and undernutrition \(^6\). While protein intake has been the main nutritional focus \(^7,8\), many older adults do not consume adequate amounts of dietary protein due to reduced energy needs, increased physical dependency, anorexia, changes in food preference, anabolic resistance, and increased inflammatory and catabolic conditions \(^9,10\).

Additionally, obesity has become an epidemic in the elderly \(^11\) and obese older adults face high risks of age-related muscle wasting such as sarcopenia \(^12,13\). In older adults with sarcopenic obesity, sarcopenia and obesity may synergistically increase their effects on physical disability, metabolic disorders, cardiovascular disease, and mortality \(^12,14-16\). Although the molecular mechanisms that underlie obesity-associated dysfunctions in lipid and glucose metabolism have been studied extensively \(^17\), the effects of obesity on the processes that regulate muscle protein metabolism are poorly understood \(^18\). Intramuscular lipids act as chemoattractants for macrophages that produce pro-inflammatory cytokines \(^19\). These cytokines not only directly contribute to the breakdown of muscle proteins \(^20\) but also interfere with the accretion of contractile material caused by chronic low-intensity muscle overloading \(^21\). Previously, Erskine and colleagues described the paradox that circulating pro-inflammatory cytokines play different roles in neuromuscular remodeling according to the age and adiposity of the individual \(^22\).
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According to the Korea National Statistical Office, the percentage of the Korean population age 65 and older is expected to rise to 24.5% by 2030 and to 38.1% by 2050 (23). Along with the rapid growth in the number of elderly individuals in Korea, sarcopenia and sarcopenic obesity have become important issues in this country. In the Korean Sarcopenic Obesity Study which included 526 healthy volunteers 20–88 years of age, the prevalence of sarcopenic obesity in older (≥60 years) men and women was 5.1% and 12.5%, respectively, as of 2008. However, there have been no Korean cohort studies on the long-term effects of dietary protein on changes in muscle mass according to the obesity status.

In the elderly, the maintenance of muscle mass and strength are critical to the abilities of an individual to survive multiple comorbidities and meet their physical demands. Therefore, we conducted a prospective cohort study of elderly Korean subjects to investigate the effects of protein intake on changes in lean mass according to the obesity status.

2. Materials and Methods

2-1 Data source and study population

Data were obtained from the large community-based cohort of the Korean Genome and Epidemiology Study (KoGES). The eligibility criteria for the participants of the KoGES at baseline included residents between the ages of 40 and 69 years who had lived in Ansan (urban) or Ansung (rural), Korea, for at least 6 months before enrolment. Baseline examinations were performed in 2001 and 2002 and follow-up examinations continued every two years until the end of 2014. Detailed information about this study has been provided elsewhere (24). Of the original 10,030 participants, 4,412 were included in the final analysis after excluding those who did not complete the baseline food frequency questionnaire (FFQ) or anthropometric data (n=2,417), who had abnormally low or high daily energy intakes (<500 or >5,000 kcal/day, n=56), who did not participate in follow-up examinations (n=3,042), or who had low skeletal muscle mass (<35.71% in men, <30.70% in women) (25) at baseline (n=103). All participants signed the written informed consent form. This study was approved by the Institutional Review Board (no. KC17ZESI0645) of Catholic Medical Center.
2-2 Assessment of usual dietary protein intake

At baseline, the participants’ usual dietary protein intake was assessed by trained dietitians using a validated 103-item semi-quantitative FFQ (26). There were nine response options for the frequency of each food (never or almost never, once per month, two or three times per month, one or two times per week, two or three times per week, three or four times per week, five or six times per week, once daily, twice daily, or three times daily) and three response options for the portion size of each food (1/2 serving, 1 serving, and ≥ 2 servings). To enhance the accuracy of recollecting serving size, pictures of each food item were used as a reference. The daily intakes of protein and other nutrients by each individual were estimated from the sum of the intakes of each food item, based on the Food Composition Database (Seoul, Korea: The Rural Development Administration, 2007). Among the 103 food items listed on the FFQ, 79 items classified as the main food sources contributing to protein intake (nine food items from meats including beef, pork, poultry, and meat products, 15 food items from fish and shellfish, three food items from soybean, four food items from milk, one food item from egg, seven food item from rice and other cereals, 27 food items from vegetables, 10 food items from noodles and breads, and three food items from potatoes) were assessed to calculate each subject’s protein intake.

2-3 Anthropometric measurements

Fat mass and lean mass (fat-free mass) were assessed using a multi-frequency bioelectrical impedance analysis (MF-BIA, InBody 3.0; Biospace, Seoul, Korea) according to standard procedures. The MF-BIA technique assumes that the human body comprises five interconnecting cylinders and measures the direct impedance in these body compartments. Using an eight-polar tactile electrode system, impedance was measured at four specific frequencies (5, 50, 250, and 500 kHz) in five segments (right arm, left arm, trunk, right leg, and left leg) for the estimation of total body water (TBW). The lean mass was estimated from the TBW and anthropometric measurements, using an algorithm for the Asian population. The fat mass was estimated by subtracting the lean mass from the total body weight. The participants fasted overnight prior to the BIA assessment. Before the examination, the researchers confirmed whether the participant had experienced intensive exercise, bathing, or excessive sweating. The body mass index (BMI) was calculated as the weight (kg) divided by the height (m) squared. Although there is no standardized definition of obesity according to fat mass, the participants were categorized into two groups by percent body fat (%BF) with reference to Asian epidemiologic studies of the %BF (normal group, %BF <25 for men and %BF <30 for women; obese group, %BF ≥25 for men and %BF ≥30 for women) (27,28).
2-4 Covariates

All lifestyle-related information was gathered by the interviewer-administered questionnaire. The questionnaire included questions on sex, age, marital status, education, income, smoking, alcohol consumption, regular physical activity, and self-perceived dental health status. Smoking status was used to classify participants into ‘smokers’ (current smoker) and ‘non-smokers’ (former smokers and non-smokers). Alcohol consumption was used to classify participants into ‘alcohol drinkers’ (current drinker) and ‘non-drinkers’ (former and never drinkers). Regular physical activity was defined as ‘yes’ if the participant performed more than 2.5 hours of exercise per week according to the global WHO recommendation (29,30). The self-perceived dental health status was categorized as poor or others (good and fair). Chronic diseases were measured by participants’ self-report of the presence or absence of 10 chronic conditions (myocardial infarction, congestive heart failure, coronary artery disease, peripheral arterial disease, cerebrovascular disease, asthma, chronic obstructive pulmonary disease, cancer, dementia, and arthritis).

2-5 Statistical analysis

Because of the well-established sex differences in the age-related changes in lean mass and muscle strength (31), all analyses were performed separately for men and women. Protein intake was examined by the protein density method, in which nutrient intake was divided by total energy intake (in grams per 1,000 kcal) (32). The protein intake per 1,000 kcal at baseline was categorized into tertiles. The baseline characteristics of the study participants were compared with respect to the tertiles of protein intake per 1,000 kcal, using Mantel-Haenszel $\chi^2$ tests for categorical variables and linear regression analyses for continuous variables. A one-way analysis of variance was used to test for between-group differences in the percent change in body composition after a 12-year follow-up, using the lowest tertile as the reference group. Independent Student’s t-tests were used to examine the differences between older (≥50 years) and younger participants (<50 years) in terms of the percent change in body composition after a 12-year follow-up. Multiple linear regression analyses were performed to determine the relative contributions of the evaluated characteristics to the lean mass at baseline. Fat mass (kg), age (<60/≥60 years), protein intake (T1/T2/T3), marital status (married/others), education (≥college/others), income (≥3,000,000 KRW per month/others), smoking (yes/no), alcohol consumption (yes/no), regular physical activity (yes/no), self-perceived dental health status (poor/others), and chronic disease (yes/no) were included as covariates in the
model. Variables with p values <0.05 were selected during stepwise regression procedures. The subjects were categorized into the normal and obese groups as %BF for linear mixed effects models to examine the independent effect of protein intake on lean mass after 12 years of follow-up. Potential confounding variables were selected using stepwise regression procedures and entered into the models. Model 1 was adjusted for age, income, alcohol consumption, smoking, regular physical activity, and chronic disease and Model 2 was adjusted for the variables in Model 1 plus fat mass. IBM SPSS Statistics for Windows, version 24.0 (IBM, Corp., Armonk, NY, USA) was used for all statistical analyses. A two-sided p-value < 0.05 was considered statistically significant.

3. Results

Table 1 shows the characteristics of the study participants according to protein intake at baseline. The protein intakes (g/kg body weight) by tertiles 1, 2, and 3 were 0.8, 1.0, and 1.3 g in men and 0.9, 1.1, and 1.4 g in women, respectively. Both men and women with high protein intakes were significantly younger (p <0.001); were more likely to live in a city (p <0.001), have a higher educational level (p <0.001), and have a higher income (p<0.001); tended to be married (p=0.019 for men, p <0.001 for women); and tended to consume alcohol currently (p <0.001 for men, p=0.001 for women) and to be physically active (p <0.001). Smoking status was not significantly associated with protein intake. Additionally, men and women with higher protein intakes had higher intakes of energy (p <0.001) and energy from fat (p <0.001) and a lower intake of energy from carbohydrates (p <0.001). Regarding body composition, men with a high protein intake had a higher weight (p <0.001), BMI (p <0.001), fat mass (p <0.001), %BF (p <0.001), and lean mass (p <0.001) at baseline, whereas among women, only lean mass differed significantly in relation to protein intake (p=0.005).

In men and women with the highest protein intakes, dietary protein was mostly derived from meats (22.9% in men and 18.4% in women), while dietary protein was mostly derived from vegetable proteins with rice and other cereals in men and women with the lowest protein intakes (41.8% in men and 44.1% in women) (Figure 1).

Among men, the percent change in body composition after 12 years of follow-up did not differ significantly according to protein intake, whereas women with a higher protein intake had lower reductions in weight, BMI, and lean mass during the follow-up period (p <0.001, p=0.014, and p <0.001, respectively). Among both men and women, older participants (≥50 years) had a greater reduction in lean mass compared to that in the younger group (<50 years) (p <0.001; T1, T2, and T3) (Figure 2).
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Table 2 shows the results of the multiple linear regression models using a stepwise procedure for selecting variables which predicted lean mass at baseline. In men, a higher fat mass (β=0.59, p <0.001), younger age (β=3.75, p <0.001), regular physical activity (β=1.07, p <0.001), higher income (β=0.83, p=0.002), current smoking (β=0.72, p=0.002), protein intake (β=0.50; p=0.039), and the presence of chronic disease (β=-2.25, p=0.019) were associated with a higher lean mass at baseline. In women, a higher fat mass (β=0.46, p <0.001), younger age (β=2.20; p <0.001), regular physical activity (β=0.89; p <0.001), and alcohol consumption (β=0.63, p <0.001) were associated with a higher lean mass at baseline.

In the linear mixed effects models, higher protein intake at baseline was associated with a higher lean mass after 12 years of follow-up in both men (β=0.79, p=0.001; highest vs. lowest tertile) and women (β=0.28, p=0.082; highest vs. lowest tertile) after adjusting for covariates (Table 3). These associations were attenuated after additional adjustment for fat mass at baseline. In further evaluation according to obesity status, the associations were stronger in the normal-weight group (men, β=0.85, p=0.002; highest vs. lowest tertile; women, β=0.97, p <0.001; highest vs. lowest tertile) but were not detected in the obese group. Age (men, β=4.08, p <0.001; women, β=2.61, p <0.001) and regular physical activity (men, β=0.88, p=0.054; women, β=0.76, p <0.001) were significantly associated with lean mass after 12 years of follow-up, regardless of obesity status. Higher income (men, β=1.36, p=0.001; women, β=0.43, p=0.046) and alcohol consumption (men, β=1.16, p=0.005; women, β=0.56, p=0.002) in both obese men and women and smoking (β=1.82, p <0.001) and chronic disease (β=-2.42, p=0.071) in obese men were significantly associated with lean mass after 12 years of follow-up.

4. Discussion

To our knowledge, this is the first large community-based prospective cohort study in Korea to investigate the associations between protein intake and changes in lean mass according to obesity status in middle-aged individuals. The results showed that men and women with higher protein intakes had higher lean mass after 12 years of follow-up but that these differences were attenuated after additional adjustment for fat mass at baseline. The associations were stronger in the normal-weight group but were not detected in the obese group.

Women with a higher protein intake had lower reductions in lean mass during the follow-up period. In a meta-analysis of adult men and women with a mean age of 50 years or older, a higher protein intake (≥25% of the daily total energy intake or ≥1.0 g/kg/d) was associated with increased lean mass retention and fat mass loss during intentional weight loss (33). Another cohort study
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investigated the association between dietary protein and changes in lean mass and appendicular lean mass in older, community-dwelling men and women, and reported that community-dwelling older adults with the highest quintile of protein intake lost ~40% less total lean body mass and appendicular lean mass over three years compared to the loss in the group with the lowest quintile of protein intake (34). Our study showed similar results in women, not in men. In women, high protein intake was associated with smaller reductions in lean mass, weight, and BMI. However, there was no significant association in men between protein intake and changes in body composition. The Quebec longitudinal study on Nutrition as a Determinant of Successful Aging reported a stronger association between protein distribution (SD of grams of protein per meal divided by grams of total protein) and muscle strength in women than that in men (men, β=-0.44, p <0.05; women, β=-0.68, p <0.001) (35). Other studies showed that the associations between diet and grip strength (men, β=0.18, p=0.035; women, β=0.27, p <0.001) (36) or physical performance (men, β=0.995, p=0.383; women, β=0.985, p=0.091) (37) were stronger in women than those in men. Although those studies did not focus on protein intake, the authors noted that women usually had healthier diets with higher micronutrient densities compared to those in men, which may have affected women’s health outcomes such as grip strength or physical performance. In another study, however, total protein intake was related to lean mass only in men, while the protein distribution was related to lean mass in both sexes but more so in men (38). Declining muscle mass can be affected by lifestyle factors as well as age-related physiological and systemic changes in the body (7), therefore, the roles played by various lifestyle factors in age-related changes in body function should be further investigated to clarify sex-specific associations between dietary protein intake and lean mass.

Consistent with the results of previous studies, we observed a positive association between protein intake and lean mass retention, after adjusting for covariates and lean mass at baseline in both men and women. Previous cohort studies of older adults have demonstrated that the quantity of dietary protein intake is the main nutritional factor associated with preserving muscle mass and maintaining physical function (39-41). In one of these studies, compared with those in the lowest tertile of protein intake (<66 g/d), women in the highest tertile (≥87 g/d) had 5.4–6.0% higher whole body and appendicular lean mass (40). A Japanese study also reported that the consumption of seafood, dairy products, and protein-rich foods may help older adults to maintain their independence (42). There is growing evidence that protein intake levels exceeding the recommended daily allowance may benefit elderly adults by preventing or mitigating sarcopenia (9,43). A review study also concluded that a protein intake level meeting the nutritional requirements of all healthy individuals does not provide protection from age-related sarcopenia (44-46). According to data from the 2008–2012 Korean National Health and Nutrition Examination Survey, 18.8% of adults ≥60
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years of age and 34.9% of adults ≥70 years of age consumed less protein than the estimated average requirements for Koreans (40 g/day for men and 35 g/day for women), indicating that many older Korean adults may have insufficient protein intake (47). Therefore, the effects of dietary protein intake in the attenuation of age-related loss of lean mass among older adults require further investigation.

The results of the present study showed that obesity reduced the muscle preservation effects of dietary protein. Dietary protein intake had a significant effect on lean mass after the 12-year follow-up in the normal-weight group but not in the obesity group. Sarcopenia and obesity share several pathophysiological mechanisms (12) that may synergistically increase the risk of negative health outcomes (48). Moreover, the rates of physical disabilities (49,50), comorbidities (51), and mortality (52,53) are higher in sarcopenic obesity than those in sarcopenia or obesity alone. A recent cross-sectional study reported that normal weight women had 14% and 10% higher muscle quality values (i.e., leg power [watts] normalised for lower-body mineral-free lean mass [kg]) than those of overweight and obese women, respectively (54). Furthermore, fat mass was associated with functional decline and muscle weakness in elderly individuals (39,55,56). In contrast, a recent meta-analysis showed that protein supplementation combined with resistance exercise training effectively prevented age-related muscle mass attenuation and leg strength loss in the elderly, regardless of body weight (57). However, on the basis of protein supplementation alone, individuals with a mean BMI ≥30 kg/m² did not exhibit a greater change in muscle volume and handgrip strength than that in those with a mean BMI <30 kg/m². The authors concluded that protein supplementation may not prevent age-related muscle loss in obese elderly. Although little is known about the mechanisms underlying the impact of obesity on lean mass in the elderly, obesity may be accompanied by a state of chronic oxidative stress, which could promote protein breakdown and direct muscle fibers into a catabolic state that, ultimately, leads to muscle wasting (58). Moreover, in overweight elderly, ineffective autophagic mechanisms may be associated with insulin resistance due to the inhibition of protein synthesis and accumulation of misfolded proteins, thus contributing to age-related skeletal muscle loss (59). In our study, protein intake may have had less impact on the prevention of lean mass loss in elderly obese than that in elderly adults with normal weight. Further research is needed to fully understand the impact of obesity on lean mass.

Similar to the results reported by previous studies, regular physical activity was positively associated with lean mass retention in both men and women, regardless of obesity status. Recent meta-analyses have reported that exercise interventions may be beneficial in improving muscle strength and physical performance (57,60,61). A prospective cohort study concluded that a higher intake of animal protein foods alone, especially in combination with a physically active lifestyle, was
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associated with the preservation of muscle mass and functional performance in elderly individuals (62). A randomized controlled trial in elderly Japanese women with sarcopenia demonstrated the significant effects of exercise and amino acid supplementation on the enhancement of muscle strength and on the combined variables of muscle mass and walking speed or muscle mass and strength (63). The lifestyles of obese men and women may, therefore, have an important influence on the retention of lean mass.

The strengths of this study included its large cohort and long follow-up period, which enabled the investigation of the associations between protein intake from habitual diets and lean mass in Koreans. However, our study also had several limitations. BIA analysis for assessing muscle mass is a useful non-invasive method in large population-based studies; however, factors such as age, hydration status, food or beverage consumption, and exercise intensity may affect the results. To reduce the possibility of measurement errors, the participants were requested to fast before the BIA assessment and their hydration status was monitored carefully. The European Working Group on Sarcopenia in Older People suggested BIA as a portable alternative to dual-energy X-ray absorptiometry (6). Additionally, we assessed dietary protein intake only at baseline and did not determine whether the protein intake of the participants had changed over the course of the follow-up period.

5. Conclusions

In conclusion, our findings support the current evidence that higher protein intakes are beneficial in preserving lean mass. The associations between dietary protein and increased lean mass were more apparent in the normal-weight group but were not detected in the obese group. Our population-based findings may inform the development of improved healthcare programmes for the Korean elderly, with aims to preserve muscle mass and maintain functionality. However, further comprehensive investigations of the factors affecting muscle strength, functional status, and muscle mass are needed to clarify the dose-response effects in older adults of varying weight status.

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Conflict of Interest: None
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Authorship: Eunjin So performed the analyzed the data and wrote the paper; Hyojee Joung and Seul Ki Choi reviewed the manuscript; All authors have primary responsibility for the final content.

References
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Figure 1. Contribution of protein sources foods to total daily protein intake
Figure 2. % change of body composition over 12 years by tertiles of protein intake per 1,000 kcal. 

% Change = ((12-y follow-up value – baseline value)/baseline value x 100). Values are mean.

* Indicates significantly linear trend across tertiles of protein intakes: P for trend<0.05.

Among both men and women, older participants (≥50 years) had a greater reduction in lean mass, compared to the younger group (<50 years) (p<0.001; T1, T2, and T3) using the Student’s t-test.
### Table 1. Characteristics of study participants by the levels of protein intake

<table>
<thead>
<tr>
<th>Protein intake (tertiles)</th>
<th>Men (n=2,096)</th>
<th>Women (n=2,316)</th>
<th>p for trend or p</th>
<th>p for trend or p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
<td>Mean</td>
</tr>
<tr>
<td>Demographics and lifestyles at baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>51.8</td>
<td>8.5</td>
<td>49.6</td>
<td>7.6</td>
</tr>
<tr>
<td>Residential area (city, %)</td>
<td>48.1</td>
<td>75.2</td>
<td>78.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Education (≥college, %)</td>
<td>16.6</td>
<td>26.9</td>
<td>31.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Household income (≥3,000,000 KRW, %)</td>
<td>15.7</td>
<td>28.1</td>
<td>78.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Marital status (married, %)</td>
<td>95.8</td>
<td>97.0</td>
<td>98.0</td>
<td>0.019</td>
</tr>
<tr>
<td>Smoking (yes, %)</td>
<td>46.8</td>
<td>42.2</td>
<td>45.2</td>
<td>0.623</td>
</tr>
<tr>
<td>Chronic diseases (yes, %)</td>
<td>1.9</td>
<td>1.1</td>
<td>1.3</td>
<td>0.381</td>
</tr>
<tr>
<td>Multi-morbidity (n)</td>
<td>0.1</td>
<td>0.2</td>
<td>0.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Dental health status (poor, %)</td>
<td>39.7</td>
<td>40.4</td>
<td>36.0</td>
<td>0.144</td>
</tr>
<tr>
<td>Alcohol drinking (yes, %)</td>
<td>66.7</td>
<td>73.4</td>
<td>80.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Regular physical activity (yes, %)</td>
<td>11.7</td>
<td>18.0</td>
<td>23.3</td>
<td>&lt;0.001</td>
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<tr>
<td>Nutrient intake at baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal/d)</td>
<td>1,904.3</td>
<td>608.5</td>
<td>1,969.7</td>
<td>483.8</td>
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<tr>
<td>Carbohydrate (% of energy)</td>
<td>75.4</td>
<td>3.9</td>
<td>69.9</td>
<td>3.5</td>
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<tr>
<td>Fat (% of energy)</td>
<td>11.8</td>
<td>3.7</td>
<td>15.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>11.3</td>
<td>0.9</td>
<td>13.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>54.1</td>
<td>17.7</td>
<td>66.3</td>
<td>16.9</td>
</tr>
<tr>
<td>Protein (g/kg body weight)</td>
<td>0.8</td>
<td>0.3</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td>11.8</td>
<td>3.7</td>
<td>15.5</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Body composition at baseline
## Accepted manuscript

| Weight (kg) | 66.9 | 9.4 | 68.2 | 9.0 | 69.8 | 9.2 | <0.001 | 58.3 | 8.3 | 59.1 | 7.5 | 58.9 | 7.4 | 0.111 |
| Body Mass Index (kg/m^2) | 24.1 | 2.8 | 24.3 | 2.7 | 24.7 | 2.7 | <0.001 | 24.7 | 3.1 | 24.7 | 2.8 | 24.4 | 2.8 | 0.078 |
| Fat mass (kg) | 14.4 | 4.5 | 14.9 | 4.5 | 15.6 | 4.5 | <0.001 | 18.5 | 4.9 | 18.8 | 4.5 | 18.5 | 4.5 | 0.410 |
| Body fat (%) | 21.1 | 4.8 | 21.5 | 4.8 | 22.0 | 4.5 | <0.001 | 31.3 | 5.0 | 31.5 | 4.6 | 31.0 | 4.8 | 0.179 |
| Lean mass (kg) | 52.5 | 6.3 | 53.2 | 6.0 | 54.3 | 6.1 | <0.001 | 39.8 | 4.5 | 40.3 | 4.1 | 40.5 | 4.2 | 0.005 |

\(^a\) Tertiles of protein intake per 1,000 kcal at baseline.

\(^b\) P for trend was calculated from a linear regression analysis for continuous variables and Mantel-Haenszel \( \chi^2 \) for categorical variables.
Table 2. Predictors of lean mass at baseline

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Men (n=2,096)</th>
<th>Women (n=2,316)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>0.59</td>
<td>0.03</td>
</tr>
<tr>
<td>Age (≤ 60 yrs)</td>
<td>3.75</td>
<td>0.32</td>
</tr>
<tr>
<td>Regular physical activity (yes)</td>
<td>1.07</td>
<td>0.30</td>
</tr>
<tr>
<td>Income (≥ 3,000,000 KRW)</td>
<td>0.83</td>
<td>0.27</td>
</tr>
<tr>
<td>Smoking (yes)</td>
<td>0.72</td>
<td>0.23</td>
</tr>
<tr>
<td>Alcohol consumption (yes)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Protein intake&lt;sup&gt;b&lt;/sup&gt; (Tertile 3)</td>
<td>0.50</td>
<td>0.24</td>
</tr>
<tr>
<td>Chronic disease (yes)</td>
<td>-2.25</td>
<td>0.96</td>
</tr>
</tbody>
</table>

<sup>a</sup> Predictors were selected by a regression model with stepwise procedure.<br>
<sup>b</sup> Protein intake per 1,000 kcal. Variables initially included in the model were fat mass (kg), age (< 60 years/≥ 60 years), protein intake (T2), protein intake (T3), marital status (married/others), education (≥ college/others), income (≥ 3,000,000 KRW per month/others), smoking (yes/no), alcohol consumption (yes/no), regular physical activity (yes/no), self-perceived dental health status (poor/others), chronic diseases (myocardial infarction, congestive heart failure, coronary artery disease, peripheral arterial disease, cerebrovascular disease, asthma, chronic obstructive pulmonary disease, cancer, dementia, and arthritis).

- indicates that the variable was not selected during stepwise procedure due to p>0.05.
Table 3. Estimates of lean mass after a 12-year follow-up by protein intake according to obesity status at baseline

<table>
<thead>
<tr>
<th>Variables</th>
<th>Men</th>
<th>Women</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n=2,096)</td>
<td>Normal&lt;sup&gt;c&lt;/sup&gt; (n=1,574)</td>
<td>Obese&lt;sup&gt;d&lt;/sup&gt; (n=522)</td>
<td>Total (n=2,316)</td>
<td>Normal&lt;sup&gt;c&lt;/sup&gt; (n=809)</td>
<td>Obese (n=1,507)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Model 1</td>
<td>Model 2</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Intercept</td>
<td>45.12</td>
<td>37.76</td>
<td>44.18</td>
<td>48.04</td>
<td>36.82</td>
<td>28.95</td>
<td>35.28</td>
</tr>
<tr>
<td>Protein intake at baseline&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>0.13</td>
<td>-0.65</td>
<td>0.28&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.18</td>
<td>0.71**</td>
</tr>
<tr>
<td>Tertile 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.79**</td>
<td>0.35†</td>
<td>0.85**</td>
<td>0.45</td>
<td>0.28†</td>
<td>0.28*</td>
<td>0.97**</td>
<td>-0.15</td>
</tr>
<tr>
<td>Age (&lt;60yrs)</td>
<td>5.00**</td>
<td>4.59**</td>
<td>5.29**</td>
<td>4.08**</td>
<td>2.74**</td>
<td>2.83**</td>
<td>3.26**</td>
</tr>
<tr>
<td>Income (≥3,000,000 KRW)</td>
<td>1.46**</td>
<td>0.91**</td>
<td>1.49**</td>
<td>1.36**</td>
<td>0.25</td>
<td>0.32*</td>
<td>-0.07</td>
</tr>
<tr>
<td>Alcohol consumption (yes)</td>
<td>0.55**</td>
<td>0.36†</td>
<td>0.33</td>
<td>1.16**</td>
<td>0.50**</td>
<td>0.54**</td>
<td>0.31</td>
</tr>
<tr>
<td>Smoking (yes)</td>
<td>0.20</td>
<td>0.73**</td>
<td>0.23</td>
<td>1.82**</td>
<td>-0.15</td>
<td>0.04</td>
<td>-0.20</td>
</tr>
<tr>
<td>Regular physical activity (yes)</td>
<td>1.44**</td>
<td>1.06**</td>
<td>1.63**</td>
<td>0.88†</td>
<td>1.22**</td>
<td>0.93**</td>
<td>2.08**</td>
</tr>
<tr>
<td>Chronic diseases (yes)</td>
<td>-2.54**</td>
<td>-2.52**</td>
<td>-2.72**</td>
<td>-2.42†</td>
<td>-0.01</td>
<td>0.14</td>
<td>-0.04</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>0.56**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.43**</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Goodness-of-fit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>p<0.05,  **p<0.01,  †p<0.1

<sup>b</sup>Protein intake per 1,000 kcal.

<sup>c</sup>Coefficient estimates and SEs are derived from linear mixed effects models adjusted for lean mass at baseline.

<sup>d</sup>Normal: %BF < 25 for men, and %BF < 30 for women.

<sup>e</sup>Obese: %BF ≥ 25 for men, and %BF ≥ 30 for women.

Model 1 was adjusted for age, income, alcohol consumption, smoking, regular physical activity, and chronic diseases. Model 2 was adjusted for variables in Model 1 plus fat mass.