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Protein intake and episodic memory: the moderating role of the apolipoprotein E ε4 status

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Abstract

Background This study investigated the correlation between protein intake and Alzheimer's disease (AD)-related cognitive decline, particularly in episodic memory, among older adults without dementia. Furthermore, we assessed the moderating effect of apolipoprotein ε4 (APOE4) on this association and analyzed its influence on other cognitive functions beyond memory.

Methods The study involved 196 participants who underwent assessments for protein intake, cognitive performance, APOE4 genotyping, and nutritional biomarkers. Protein intake was categorized into low, medium, and high based on the consumption of dairy, legumes, eggs, meat, and fish.

Results High protein intake was significantly associated with better episodic memory and overall cognition. Moreover, a significant interaction was found between high protein intake and APOE4, indicating that APOE4 moderates the association between high protein intake level and episodic memory. Sensitivity analysis confirmed these results among participants with stable food intake.

Conclusions Our study results demonstrated that high protein intake is associated with better episodic memory among older adults without dementia. Furthermore, the findings highlight the significant role of APOE4 status in moderating the relationship between protein consumption and episodic memory. These results suggest that dietary interventions focusing on protein intake could be beneficial for cognitive health, particularly in individuals with a genetic predisposition to AD.

Keywords Protein intake, Cognition, APOE4, Episodic memory, Alzheimer's disease

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Background

Alzheimer's disease (AD), a widespread neurodegenerative condition in older adults, is characterized primarily by a gradual decline in cognitive function. [1] This decline often starts years before the development of dementia, making cognitive decline the foremost clinical feature of AD [2]. Many studies have demonstrated a consistent decline in episodic memory over the course of AD, spanning the preclinical, prodromal, and early stages. These findings indicate that episodic memory is typically the initial cognitive domain affected by AD. [3–6] Episodic memory, which refers to the ability to recollect specific events and experiences in time (e.g., remembering a birthday party or a family vacation), is fundamental for daily functioning. Its decline can significantly impair quality of life and autonomy, impacting an individual's ability to perform everyday tasks independently [7].

Though there are longstanding and novel pharmacological interventions that can help improve cognitive and functional symptoms of AD (e.g., donepezil, memantine, lecanemab), there is yet to be a treatment that prevents or reverses the impact of AD on the brain. As such, evidence-based approaches focusing on lifestyle modifications, such as nutritional or dietary interventions, may help prevent or mitigate the decline in episodic memory and other cognitive functions related to AD [8]. Furthermore, such dietary adherence is also associated with AD biomarkers, which are precursors to cognitive decline and memory issues [9].

Proteins are vital nutrients that play a critical role in maintaining normal body functions. They are the fundamental components of muscles and organs and are indispensable for tissue and cell repair, as well as the synthesis of neurotransmitters [10–13]. Additionally, they may exert neuroprotective effects, possibly by mitigating oxidative stress and inflammation within the brain, both of which are significant contributors to AD or related cognitive decline [11–13]. In animal studies, diets low in calories and protein have been linked to extended lifespans and improved outcomes related to aging, [10, 14] while high protein intake has been reported to be associated with an increased risk of cardiovascular diseases [15]. In contrast, studies in humans, particularly among older populations, suggest that inadequate protein intake may raise the risk of sarcopenia and frailty [16]. These conditions are closely associated with cognitive impairments [17]. Therefore, while reducing protein intake might benefit longevity based on animal models, ensuring sufficient protein consumption could be crucial for maintaining muscle mass and cognitive function in older adults.

However, epidemiological studies on protein intake and cognitive decline have yielded mixed results [18–20]. A previous study reported that high total protein intake could be related to a greater risk of cognitive decline, [19] while other studies, like the Nurses' Health Study and the Health Professionals Follow-up Study, indicate it may reduce the likelihood of subjective cognitive decline [18]. Yet, the latter did not employ objective cognitive assessments, highlighting the complexity and need for further study in this area.

Apolipoprotein ε4 (APOE4), known as the highest-risk gene for AD [21], promotes the development of amyloid-beta (Aβ) pathology in older adults carrying the gene [21, 22]. It may also compromise several brain homeostasis pathways, leading to cognitive impairments and the onset of dementia [21, 23–25]. To examine the intricate connection between dietary protein intake and cognitive function more thoroughly, a notable cohort study has examined how genetic factors might influence this dynamic [20]. The study found no significant overall association between total or fish protein intake and cognitive function. Intriguingly, however, it revealed a significant interaction with the APOE4 allele, a genetic variant known to elevated the risk of AD. Specifically, participants with the APOE4 allele who had high total or fish protein intake showed significantly lower odds of cognitive decline, a pattern not observed in those without this allele [20]. This suggests that genetic predispositions can modulate the impact of diet on cognitive function. Yet, the study's reliance on telephone-based cognitive assessments raises concerns about the potential lack of precision compared to face-to-face evaluations, due to the inherent limitations such as the inability to observe visual cues and the potential for environmental distractions to influence test results [26].

In light of this, researchers have yet to thoroughly investigate the association between total protein intake and AD-specific cognition, including whether the APOE4 allele influences this relationship, especially through more reliable face-to-face assessments. Recognizing this gap, we investigated using face-to-face assessments to explore the relationship between total protein intake and cognition specific to AD, particularly the decline in episodic memory, in older adults without dementia. Subsequently, we explored how APOE4 status influences this relationship and examined the effect of total protein intake on non-memory cognitive functions to provide a comparative analysis.

Methods

Participants

This study is a component of the General Lifestyle and AD (GLAD) study, an ongoing prospective cohort investigation that started in 2020. As of September 2022, a total of 227 individuals had volunteered for the assessment of eligibility for the GLAD study. Among them, 196 older adults without dementia, aged between 65 and 90

years—comprising 113 cognitively normal (CN) adults and 83 adults with mild cognitive impairment (MCI)—were enrolled in the GLAD study. However, 31 individuals were excluded for various reasons: 9 individuals met one of the exclusion criteria, such as major psychiatric disorders (e.g., schizophrenia, bipolar disorder, major depressive disorder, alcohol/substance abuse or dependence, delirium) ($n=3$), significant neurological or medical conditions affecting cognitive function (e.g., stroke, Parkinson's disease) ($n=4$), illiteracy, major visual or hearing impairments, severe communication or behavioral problems (e.g., severe speech or language disorders, non-compliance with study protocols) that complicate clinical assessments ($n=1$), or were undergoing treatment with investigational drugs ($n=1$). Additionally, 2 participants were excluded for not fitting any diagnostic categories, and 20 participants were removed from the study due to withdrawal of consent ($n=17$) or loss of contact ($n=3$).

Participants were recruited from a dementia screening program held at the memory clinic of Hallym University Dongtan Sacred Heart Hospital in Hwaseong, South Korea. Eligibility for study participation was determined through screening processes. Additional recruitment efforts reached out into the community, utilizing referrals from current participants, as well as their families, friends, and acquaintances. The CN group comprised individuals who scored 0 on the Clinical Dementia Rating, [27] indicating no presence of MCI or dementia. Individuals exhibiting a score of 0.5 on the Clinical Dementia Rating and meeting the criteria for amnestic MCI were categorized as MCI. This includes having memory complaints confirmed by an informant, measurable objective memory impairment, preserved general cognitive functions, the capacity to carry out daily activities independently, and no dementia diagnosis. Objective memory impairment was determined by age-, sex-, and education-adjusted z-scores below −1.0 on any of the four episodic memory tasks in the Korean version of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuropsychological battery [28–30], which includes word list memory, word list recognition, word list recall, and constructional recall tests.

Standard protocol approvals, registrations, and participants consent

The Institutional Review Board of Hallym University Dongtan Sacred Heart Hospital approved the study protocol, ensuring compliance with the Declaration of Helsinki. Informed consent was obtained from all participants before the study began.

Clinical assessments

The participants underwent comprehensive clinical evaluations led by experienced psychiatrists, with the CERAD clinical and neuropsychological battery [28–30] forming the core of these assessments, conducted by specialized neuropsychologists. The focus was primarily on episodic memory, recognized as the initial cognitive domain to manifest changes in AD, [3–6] juxtaposed against non-memory cognitive functions. An episodic memory score was calculated by summing the raw scores of four specific tests within the CERAD neuropsychological battery (scoring range: 0–61): word list memory (scoring range: 0–30), word list recognition (scoring range: 0–10), word list recall (scoring range: 0–10), and constructional recall (scoring range: 0–11). Conversely, the non-memory cognition score was derived by summing the raw scores of three distinct tests (scoring range: 0–50): verbal fluency (scoring based on the number of correct words generated), a modified version of the Boston naming test (scoring range: 0–15), and constructional praxis (scoring range: 0–11). The overall cognitive performance was based on the total score (TS) derived by summing the raw scores of seven CERAD tests, except VF for which maximum score of 24 was set for the purpose of TS based on the manual (scoring range: 0-111), incorporating both memory and non-memory assessments [31]. In addition, skilled researchers conducted thorough interviews with participants and their families to evaluate vascular risk factors such as hypertension, diabetes mellitus, dyslipidemia, coronary heart disease, transient ischemic attack, and stroke. A vascular risk score (VRS) was then computed by assigning one point for each present these vascular risk factor, resulting in a composite score ranging from 0 to 6, expressed as a percentage of the maximum possible score [32]. The Geriatric Depression Scale (GDS) was utilized to evaluate depressive symptoms in the participants [33, 34]. Economic status was categorized into three levels based on annual income in relation to the minimum cost of living (MCL) as defined by the Ministry of Health and Welfare, Republic of Korea, with benchmarks established in November 2012 (http://www.law.go.kr). For a single-person household, the MCL was 572,168 Korean Won (approximately US$ 507.9) per month, increasing by 286,840 Korean Won (around US$ 254.6) monthly for each additional family member. To measure physical activity levels, the Korean version of the Physical Activity Scale for the Elderly (PASE) was used, [35, 36] which assesses the intensity of leisure, frequency, and duration, household, and occupational activities over the past week. The PASE score, combining these activity areas, served as an indicator of the participants' overall physical activity. A higher PASE score indicates more physical activity, while a lower score indicates less

physical activity. The accuracy of the data was verified through interviews with reliable informants.

Assessment of protein intake

The Mini Nutritional Assessment (MNA), a comprehensive tool developed to assess the nutritional status of elderly individuals, was used in the interviews to investigate dietary protein intake [37]. It includes questions with three-month assessment period that cover four main areas: anthropometric measurements, dietary intake, global assessment, and self-perceived health and nutrition [37, 38]. In our study, we utilized the long-version MNA, which has been extensively validated and shown to be a reliable measure for assessing nutritional status in elderly populations [37, 38]. While some items in the MNA focus on changes over the past three months (e.g. decreased food intake), others, like protein intake and fruit and vegetable consumption, assess the current nutritional status [37, 38]. While the MNA may not provide the precision of methods like 24-hour dietary recalls or dietary diaries, it has shown moderate correlation with these detailed assessments [38]. Additionally, the MNA's effectiveness in evaluating protein intake has been validated in studies that demonstrate its ability to identify low protein intake and its association with health outcomes such as sarcopenia, frailty, and cognitive decline [37, 39].

Protein intake levels were classified as 'low', 'medium', or 'high' based on adherence to three key consumption markers: daily dairy, weekly legumes or eggs (two or more servings), and daily meat, fish, or poultry. Levels were defined as low (0–1 markers met), medium (2 markers met), and high (all 3 markers met) [37].

Assessment of other dietary patterns

In the interviews conducted using the MNA [37], we also examined other dietary patterns, including the types of food consumed such as fruits and vegetables, as well as the participants' nutritional status. Included in this were any modifications in dietary intake over the previous three months caused by issues like diminished appetite, gastrointestinal troubles, or difficulties in chewing and swallowing.

Blood test

Blood samples were obtained via venipuncture after an overnight fast, specifically between 8 and 9 a.m. in the early morning. The levels of albumin, glucose, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol were measured utilizing a COBAS c702 analyzer along with specialized reagents from Roche Diagnostics (Mannheim, Germany).

APOE4 genotyping

Using EDTA anticoagulated vacutainer tubes, blood samples were collected, and DNA extraction was performed with the QIAamp DSP DNA Blood Mini Kit and QIAcube HT System from QIAGEN in Hilden, Germany. APOE genotyping was conducted using the Seeplex ApoE ACE Genotyping Kit (Seegene, Seoul, Korea) and the ProFlex PCR system (ThermoFisher Scientific, Waltham, USA), following the specified protocols. Capillary electrophoresis (QIAxcel Advanced System, QIAGEN, Hilden, Germany) was used to analyze the PCR products. Genotypes were determined as ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, or ε4/ε4 based on the electrophoresis results and the manufacturer's guidelines. Individuals possessing at least one ε4 allele were classified as APOE4-positive.

Statistical analyses

To explore the link between protein intake and cognitive function, we performed multiple linear regression analyses with protein intake levels as the independent variable and cognitive function as the dependent variable. Low protein intake level was used as the reference category. Recognizing the influence of various factors on cognitive performance, we considered potential confounders such as age, sex, APOE4 status, education, clinical diagnosis, vascular risk factors, levels of depression, annual income, overall physical activity, dietary habits (specifically fruit and vegetable intake), and blood nutritional markers (like glucose, HDL, and LDL cholesterol).

We used two models to adjust for covariates step-by-step. The first model included age, sex, APOE4 status, education, and clinical diagnosis. The second model added VRS, GDS score, annual income, PASE total score, dietary habits, and blood nutritional markers. To confirm the robustness of our regression analyses, we assessed the assumptions of normality and homoscedasticity of residuals, and verified the absence of collinearity by utilizing normal probability plots, scatter plots, and variance inflation factor (VIF) values. Additionally, we examined the moderating effect of certain covariates (age, sex, APOE4 status, education, and clinical diagnosis) by incorporating two-way interaction terms in our multiple linear regression analyses. When significant interactions were found, we conducted further linear regression analyses to delve deeper into these effects. For sensitivity analyses, we repeated our analyses with participants who had not reported decrease in food intake over the past three months due to decrease in appetite, problem of digestion, or difficulties with chewing/swallowing. These were done to remove the potential impact of physical or mental health conditions on both protein intake levels and cognitive function. A *GPower analysis (G*Power 3.1.9.6 software) was conducted to ensure adequate power for detecting significant effects, using an effect size (f^2) of

0.15 (medium effect size), alpha level of 0.05, power (1-β) of 0.90, and 10 predictors (including 3 main predictors for protein intake levels, 5 covariates, and 2 interaction terms). The analysis indicated that a minimum sample size of 147 participants was required. Our study includes 196 participants, exceeding this requirement and ensuring sufficient power. All statistical analyses were performed using SPSS software (version 28.0; IBM Corp, Armonk, NY, USA).

Results

Participant characteristics

The demographic and clinical characteristics of the study participants are outlined and classified according to their protein intake levels in Table 1. Among the 196 participants, the distribution across the protein intake categories was as follows: 92 participants were classified within the low protein intake group, 73 were allocated to the medium protein intake group, and the remaining 31 participants fell into the high protein intake group. Notably, those with higher protein intake performed better on the mini-mental status examination (MMSE) and total, memory, and non-memory composite scores, had higher income, and had better HDL levels. None of the study participants were malnourished, i.e., serum albumin < 3.5 g/dL [40].

Association between protein intake levels and cognition

The high protein intake group demonstrated greater episodic memory (β = 0.147, $p = 0.004$) compared to the low protein intake group, although no significant difference was observed in non-memory cognition, even after

Table 1 Baseline participant characteristics according to protein intake categories

Characteristic	Protein intake				
	Total	Low	Medium	High	*p*
N	196	92	73	31	
Age, y	72.65 (5.95)	73.65 (5.88)	71.80 (5.91)	71.68 (6.00)	0.086[a]
Female, No. (%)	138 (70.41)	64 (69.57)	51 (69.86)	23 (74.19)	0.880[b]
Education, No. (%)	9.62 (4.51)	9.30 (4.67)	9.49 (4.02)	10.87 (5.01)	0.236[a]
MMSE	25.58 (3.45)	24.93 (3.97)	25.62 (2.98)	27.42 (1.88)	0.002[a]
APOE4 positivity, No. (%)	40 (20.41)	16 (17.39)	14 (19.18)	10 (32.26)	0.196[b]
Clinical diagnosis, CN, No. (%)	113 (57.65)	52 (56.52)	40 (54.79)	21 (67.74)	0.453[b]
VRS, %	23.98 (18.58)	26.99 (19.27)	22.15 (18.44)	19.35 (15.57)	0.079[a]
GDS score	10.92 (7.24)	11.47 (7.15)	10.92 (7.50)	9.29 (6.88)	0.353[a]
PASE total score	64.77 (46.21)	68.75 (49.22)	63.84 (45.07)	55.16 (38.80)	0.360[a]
Annual income, No. (%)					0.004[b]
< MCL	25 (12.75)	17 (18.48)	7 (9.59)	1 (3.23)	
≥ MCL, < 2 × MCL	62 (31.63)	29 (31.52)	29 (39.73)	4 (12.90)	
≥2 × MCL	109 (55.61)	46 (0.50)	37 (50.68)	26 (83.87)	
Decrease in food intake over the past three months					0.368[b]
no, n (%)	182 (92.86)	83 (90.22)	70 (95.89)	29 (93.55)	
yes, n (%)	14 (7.14)	9 (9.78)	3 (4.11)	2 (6.45)	
Fruit or vegetables, n (%)					0.679[b]
high	119 (60.71)	55 (59.78)	43 (58.90)	21 (67.74)	
low	77 (39.29)	37 (40.22)	30 (41.10)	10 (32.26)	
Serum nutritional markers					
Glucose, fasting, mg/dL	108.15 (19.94)	110.53 (20.88)	104.74 (14.41)	109.23 (27.01)	0.173[a]
HDL-Cholesterol, mg/dL	54.64 (12.96)	51.91 (12.75)	56.38 (13.09)	58.70 (11.80)	0.015[a]
LDL-Cholesterol, mg/dL	96.40 (33.82)	95.31 (35.07)	97.85 (31.52)	96.23 (36.34)	0.893[a]
Malnutrition, No. (%)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	
CERAD-NP					
Total score	69.98 (15.61)	66.66 (14.38)	68.45 (14.46)	83.42 (15.13)	< 0.001[a]
Episodic memory score	35.10 (9.48)	33.66 (9.27)	33.67 (8.91)	42.71 (7.90)	< 0.001[a]
Non-memory score	34.25 (6.62)	33.22 (6.86)	33.96 (6.10)	38.00 (6.62)	0.002[a]

MMSE mini-mental status examination, APOE4 apolipoprotein ε4, CN cognitively normal, MCL minimum cost of living, VRS vascular risk score, GDS geriatric depression scale, PASE physical activity scale for the elderly, MCL, minimum cost of living, CERAD-NP, consortium to establish a registry for Alzheimer disease neuropsychological battery

Data are expressed as mean (standard deviation), unless otherwise indicated

[a] by one-way analysis of variance

[b] by chi-square test

Table 2 Results of multiple linear regression analyses for the associations between protein intake categories and cognition

	Total score		Episodic memory score		Non-memory score	
	β †	*p*	β †	*p*	β †	*p*
Overall						
Model 1[a]						
High	0.236	< 0.001	0.189	< 0.001	0.125	0.087
Medium	0.047	0.849	< 0.001	0.997	0.039	0.575
Low	-		-		-	
Model 2[b]						
High	0.197	< 0.001	0.147	0.004	0.073	0.311
Medium	0.022	0.693	-0.031	0.520	0.005	0.934
Low	-		-		-	

APOE4 apolipoprotein ε4, VRS vascular risk score, GDS geriatric depression scale

† By multiple linear regression analysis (low protein intake served as the reference group)

[a] Adjusted for age, sex, apolipoprotein ε4, education, and clinical diagnosis

[b] Adjusted for age, sex, apolipoprotein ε4, education, clinical diagnosis, VRS, GDS score, annual income, physical activity, fruit/vegetable, fasting glucose, and HDL- or LDL- cholesterol

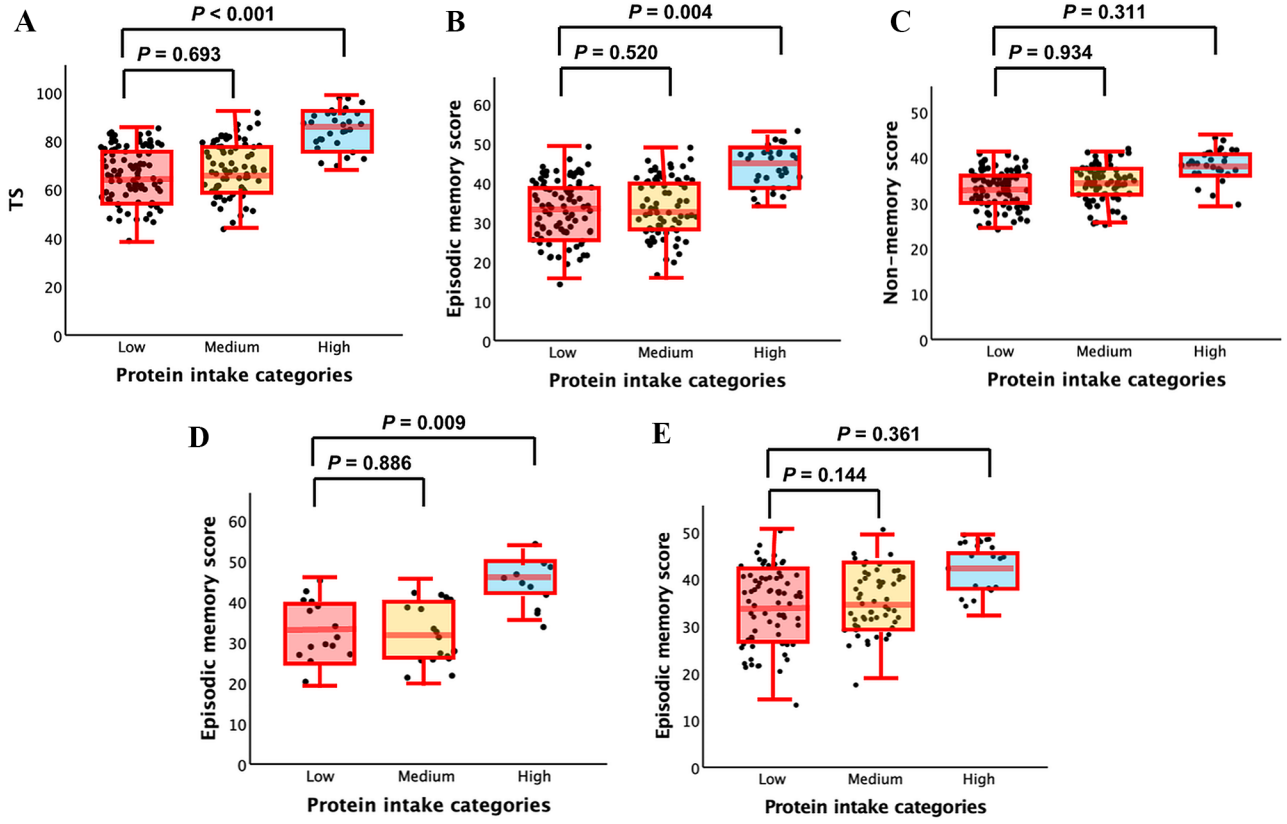

Fig. 1 Plots of the associations between protein intake and cognition (**A**-**E**): (**A**) protein intake categories vs. total score (TS), (**B**) protein intake categories vs. episodic memory score, (**C**) protein intake categories vs. non-memory score, (**D**) protein intake categories vs. episodic memory score in APOE4-positive status, and (**E**) protein intake categories vs. episodic memory score in APOE4-negative status. (**A**–**E**) were adjusted for potential covariates; mean cognition values are presented and error bars represent standard error

adjusting for confounding factors [Table 2; Fig. 1(B) and 1(C)]. The high protein intake group also showed better overall cognition (β=0.197, p<0.001) compared to the low protein intake group [Table 2; Fig. 1(A)].

Moderation of the association between protein intake levels and cognition

A significant interaction was found between high protein intake and APOE4 (β=0.146, p=0.018), indicating that APOE4 moderates the association between high protein intake level and episodic memory (Table 3). In contrast, age and sex were also significant factors, with older age

Table 3 Moderating effects of age, sex, APOE4, and clinical diagnosis on the association between each of protein intake categories and cognition

	Total score		Episodic memory score		Non-memory score	
	β †	*p*	β †	*p*	β †	*p*
Model for age effect						
Protein intake categories						
High	0.372	0.573	0.382	0.506	-0.646	0.419
Medium	-0.556	0.411	0.012	0.983	-0.756	0.357
Low	-		-		-	
Age	-0.290	< 0.001	-0.264	< 0.001	-0.253	0.009
Protein intake categories × Age						
High × Age	-0.142	0.829	-0.201	0.725	0.751	0.345
Medium × Age	0.577	0.389	-0.040	0.945	0.750	0.356
Low × Age	-		-		-	
Model for sex effect						
Protein intake categories						
High	0.309	< 0.001	0.213	< 0.001	0.105	0.199
Medium	-0.001	0.989	-0.067	0.247	-0.008	0.918
Low	-		-		-	
Sex	0.167	0.023	0.129	0.045	0.129	0.154
Protein intake categories × Sex						
High × Sex	-0.114	0.080	-0.081	0.156	-0.132	0.092
Medium × Sex	0.019	0.804	0.076	0.261	-0.012	0.900
Low × Sex	-		-		-	
Model for APOE4 effect						
Protein intake categories						
High	0.165	0.014	0.084	0.143	0.030	0.716
Medium	0.010	0.871	-0.050	0.336	-0.008	0.917
Low	-		-		-	
APOE4	-0.078	0.322	-0.108	0.111	-0.042	0.662
Protein intake categories × APOE4						
High × APOE4	0.073	0.307	0.146	0.018	0.100	0.253
Medium × APOE4	0.038	0.611	0.062	0.329	0.043	0.637
Low × APOE4	-		-		-	
Model for clinical diagnosis effect						
Protein intake categories						
High	0.252	< 0.001	0.159	0.006	0.147	0.067
Medium	-0.049	0.525	-0.105	0.119	-0.036	0.701
Low	-		-		-	
Clinical diagnosis	-0.457	< 0.001	-0.630	< 0.001	-0.205	0.025
Protein intake categories × Clinical diagnosis						
High × Clinical diagnosis	-0.066	0.292	0.022	0.685	-0.096	0.207
Medium × Clinical diagnosis	0.117	0.194	0.127	0.105	0.062	0.569
Low × Clinical diagnosis	-		-		-	

APOE4 apolipoprotein ε4, GDS geriatric depression scale, VRS vascular risk score

† By multiple logistic regression analysis controlling for age, sex, apolipoprotein ε4, education, clinical diagnosis, VRS, GDS score, annual income, physical activity, fruit/vegetable, fasting glucose, and HDL- or LDL- cholesterol as covariates when appropriate

and sex, being associated with worse performance on tests of episodic memory and cognition. However, unlike APOE4 status, no moderating effect on the association between protein intake and cognition was observed for age and sex.

Further subgroup analyses showed that high protein intake level was significantly associated with better episodic memory only in the APOE4-positive subgroup (β=0.443, p=0.009), not in the APOE4-negative one [Table 4; Fig. 1(D) and 1(E)]. On the other hand, there were no significant interactions between protein intake levels and variables like age, sex, and clinical diagnosis (Table 3).

Sensitivity analyses

Older individuals who maintained consistent food intake over the past 3 months showed cognitive performance comparable to that of the entire cohort (Table 5).

Discussion

In this study of older adults without dementia, participants with high protein intake demonstrated greater episodic memory and overall cognition, but not in non-memory cognitive functions, compared to those with low protein intake. Interestingly, the APOE4 status influenced the link between high protein intake and episodic memory, with APOE4 carriers showing a positive correlation, which was not observed in non-carriers. These results indicate that dietary protein, APOE4 status, and AD or related cognitive declines may interact in significant ways.

In this study, we explored the association between high protein intake and episodic memory, aligning with prior research that suggests high protein consumption may safeguard against conditions like sarcopenia and frailty, which are linked to cognitive decline.[14,15] This connection extends to subjective cognitive decline as well, highlighting the potential cognitive benefits of dietary protein [18]. Despite these findings, the relationship between total protein intake and cognitive decline remains ambiguous in epidemiological human studies [18–20, 41]. For instance, one cohort study found no significant link between total or fish protein intake and cognitive decline, while another associated high total protein consumption with an increased risk of cognitive decline, as measured by brief telephone-based cognitive assessments [19]. However, these assessments might be less accurate and reliable due to their inherent limitations, such as the lack of observational depth, absence of visual cues and body language, and susceptibility to environmental distractions. Studies have shown that telehealth assessments can have reduced validity compared to face-to-face assessments in certain contexts, particularly for nuanced cognitive evaluations [42]. One meta-analysis study

Table 4 Results of multiple linear regression analyses for the associations between protein intake categories and cognition according to APOE4 status

	Total score		Episodic memory score		Non-memory score	
	β †	*p*	β †	*p*	β †	*p*
APOE4-positive						
Model 1[a]						
High	0.523	0.001	0.547	< 0.001	0.352	0.059
Medium	0.155	0.230	0.123	0.318	0.172	0.281
Low	-		-		-	
Model 2[b]						
High	0.415	0.011	0.463	0.009	0.180	0.272
Medium	0.007	0.958	0.022	0.886	-0.027	0.855
Low	-		-		-	
APOE4-negative						
Model 1[a]						
High	0.137	0.029	0.073	0.166	0.022	0.780
Medium	-0.017	0.774	-0.071	0.158	-0.025	0.735
Low	-		-		-	
Model 2[b]						
High	0.115	0.064	0.048	0.361	-0.006	0.933
Medium	-0.016	0.784	-0.073	0.144	-0.028	0.701
Low	-		-		-	

APOE4 apolipoprotein ε4, VRS vascular risk score, GDS geriatric depression scale

† By multiple linear regression analysis (low protein intake served as the reference group)

[a] Adjusted for age, sex, apolipoprotein ε4, education, and clinical diagnosis

[b] Adjusted for age, sex, apolipoprotein ε4, education, clinical diagnosis, VRS, GDS score, annual income, physical activity, fruit/vegetable, fasting glucose, and HDL- or LDL- cholesterol

Table 5 Results of multiple linear regression analyses for the associations between protein intake categories and cognition in old adults with no decrease in food intake over the past 3 months (n = 182)

	Total score		Episodic memory score		Non-memory score	
	β †	*p*	β †	*p*	β †	*p*
Overall						
Model 1[a]						
High	0.243	< 0.001	0.192	< 0.001	0.120	0.107
Medium	0.083	0.142	0.032	0.521	0.071	0.319
Low	-		-		-	
Model 2[b]						
High	0.218	< 0.001	0.161	0.002	0.085	0.259
Medium	0.068	0.276	0.002	0.970	0.045	0.528
Low	-		-		-	
APOE4-positive						
Model 1[c]						
High	0.483	0.002	0.510	< 0.001	0.323	0.081
Medium	0.112	0.392	0.091	0.473	0.125	0.445
Low	-		-		-	
Model 2[d]						
High	0.401	0.017	0.442	0.014	0.233	0.161
Medium	-0.017	0.909	-0.001	0.993	-0.043	0.783
Low	-		-		-	
APOE4-negative						
Model 1[c]						
High	0.154	0.015	0.081	0.113	0.031	0.703
Medium	0.029	0.635	-0.033	0.509	0.020	0.796
Low	-		-		-	
Model 2[d]						
High	0.144	0.028	0.064	0.221	0.012	0.881
Medium	0.033	0.595	-0.034	0.494	0.022	0.785
Low	-		-		-	

APOE4 apolipoprotein ε4, VRS vascular risk score, GDS geriatric depression scale

† By multiple linear regression analysis (low protein intake served as the reference group)

[a] Adjusted for age, sex, apolipoprotein ε4, education, and clinical diagnosis

[b] Adjusted for age, sex, apolipoprotein ε4, education, clinical diagnosis, VRS, GDS score, annual income, physical activity, fruit/vegetable, fasting glucose, and HDL- or LDL- cholesterol

[c] Adjusted for age, sex, education, and clinical diagnosis

[d] Adjusted for age, sex, education, clinical diagnosis, VRS, GDS score, annual income, physical activity, fruit/vegetable, fasting glucose, and HDL- or LDL- cholesterol

found no strong association between meat consumption and cognitive disorders, with most studies showing non-significant results and some indicating a protective effect of regular meat intake [41]. In contrast, our study demonstrated a significant positive association between high overall protein intake from diverse sources and better episodic memory. The potential reasons for mixed results in the literature could include variations in protein intake measurement, differences in sample characteristics, and the stage of AD in participants. Conversely, data from the Nurses' Health Study and the Health Professionals Follow-up Study suggested that high total protein intake could be linked to reduced odds of subjective cognitive decline [18]. Notably, this study did not incorporate objective cognitive assessments, which could have provided a more comprehensive understanding of the cognitive implications of protein intake. Our study addresses these methodological limitations by employing a more rigorous approach to evaluating cognitive function. We utilized a comprehensive battery of objective cognitive tests administered face-to-face, thereby enhancing the reliability and depth of our cognitive assessments. Through this refined methodology, we have identified a significant association between high protein intake and better episodic memory performance. This finding contributes to the ongoing discourse on dietary influences on cognitive health, suggesting that protein intake may play a crucial role in maintaining cognitive function, particularly in aging populations.

It is indeed interesting that our human study results contrast with existing animal study findings [10, 14] regarding the associations between protein intake and

health. One reason for these discrepancies is the role of the APOE4 gene. In humans, the APOE4 allele is linked to higher risks of AD and cardiovascular issues, influencing protein intake's effects on health [43]. In animal models, the APOE4 gene's impact differs significantly, making it challenging to replicate human conditions accurately [44]. Additionally, variations in study design contribute to these inconsistent findings. Human studies include diverse populations, enhancing real-world applicability, while animal studies use controlled environments with genetically similar subjects [45]. Metabolic and physiological differences between species further complicate the comparison [46]. These factors highlight the need for more standardized and comprehensive research to better understand protein intake's impact on health across species. Future research should address genetic variations, improve study designs, and use animal models that closely mimic human physiology.

Our study also examined how the APOE4 allele affects the association between total protein and cognitive decline, focusing on its specific effect on episodic memory compared to other cognitions. This focus is crucial, as the decline in episodic memory is often the first sign of AD progression [3–5, 47–49]. The specific way in which the APOE4 allele impacts episodic memory highlights its potential importance in early detection and understanding of AD. A cohort study observed significant interaction of total protein or fish protein intake with APOE4, indicating that high total protein intake and fish protein intake were both significantly associated with lower odds of cognitive decline among the participants with APOE4 allele, but not among those without APOE4 allele [20]. However, the study's reliance on telephone-based cognitive assessments introduces limitations, particularly concerning the accuracy of cognitive evaluations in older adults. To overcome these limitations, our study used detailed, in-person assessments of cognitive function. Through this approach, we found that the APOE4 allele significantly affects the relationship between high dietary protein intake and episodic memory improvement. Specifically, a strong link between high protein intake and better episodic memory was seen only in individuals with the APOE4 allele, not in those without it. This clear insight into how genetics can influence the effects of high-protein diet on AD-related cognitive decline not only elucidates understanding of the complex relationship between genetics, high-protein diet, and AD-related cognitive decline but also suggests the need for personalized high-protein diet advice. Such personalized high-protein diet advice could enhance AD-related cognitive function in older adults, especially those at genetic risk for AD, offering a new way to use high-protein diet as a tool to fight against the AD-related cognitive decline.

It is interesting to consider why age and sex did not moderate the effects of protein intake on cognitive functioning, while APOE4 status did. APOE4 has a well-established biological mechanism affecting lipid metabolism and amyloid-beta deposition, directly impacting cognitive decline and AD progression. [43, 50, 51] Protein intake may interact with these pathways differently than with general aging or sex differences. Age and sex, although influential on cognitive performance, may not interact with dietary protein intake in the same direct manner. Older adults face various age-related factors (e.g., overall health, comorbidities) that can overshadow the benefits of higher protein intake on cognitive function [52]. Similarly, sex differences in cognitive decline involve hormonal, social, and lifestyle factors, making the interaction with dietary protein less consistent [53]. This suggests that the moderating effect of protein intake on cognitive function is more specifically linked to genetic factors like APOE4 status rather than broader demographic factors. Further investigation is needed to understand this hypothesis fully.

The underlying mechanism linking high protein intake to the prevention of AD or related cognitive decline is multifaceted, potentially involving the dysfunction of critical biological pathways that are central to AD pathogenesis [10–12]. The protective role of dietary protein in AD can be attributed to its essential functions in maintaining neuronal integrity, repairing tissue, and synthesizing neurotransmitters vital for cognitive processes [10–12]. Adequate protein consumption facilitates neuroplasticity, pivotal for memory and learning, and influences the regulation of brain-derived neurotrophic factor (BDNF), a key player in maintaining cognitive health [11, 12, 54]. Furthermore, certain amino acids present in proteins may exert neuroprotective effects, possibly by mitigating oxidative stress and inflammation within the brain, both of which are significant contributors to AD or related cognitive decline [11–13].

For individuals carrying the APOE4 allele, the beneficial impact of high protein intake against cognitive decline is notably pronounced, likely due to the interaction between dietary proteins and lipid metabolism [25]. APOE4 adversely affects lipid and cholesterol transport in the brain, thereby influencing amyloid-beta deposition and clearance [21, 23–25]. A diet high in protein may offset the detrimental effects of APOE4 on lipid metabolism, reducing amyloid accumulation. This mechanism is particularly relevant for APOE4 carriers, who face an increased risk of AD [21, 23–25, 55, 56]. The dietary proteins' engagement with APOE4 could also modulate other critical pathways, including neuronal repair and inflammation, underscoring a more pronounced protective effect against cognitive decline [25, 55, 56].

In our study, we conducted a comprehensive clinical assessment, including an analysis of protein intake levels (type, frequency, and quantity), laboratory blood tests, nutritional markers, and multiple cognitive domain tests. To explore the link between high protein intake and AD-related cognitive decline, we utilized statistical models to account for potential confounders. Notably, higher protein intake was associated with better episodic memory even when controlling for variables such as age, sex, APOE4 status, education, clinical diagnosis, vascular risk factors, levels of depression, annual income, overall physical activity, dietary habits (specifically fruit and vegetable intake), and blood nutritional markers (like glucose, HDL, and LDL cholesterol). This finding highlights the potential cognitive benefits of higher protein intake in the context of AD. However, this study has several limitations. Firstly, being cross-sectional, it restricts our ability to determine bi-directionality and limits causal inferences. To establish the potential benefits of high protein intake on AD-related cognitive function, the findings need to be replicated in well-powered prospective or trial studies. Second, we did not extensively evaluate the impact of other dietary components or overall dietary patterns that might influence cognitive health while we focused on total protein intake. Instead, we attempted to mitigate the influence of other foods by examining the intake of fruits and vegetables, which are known to benefit cognitive function. Third, the association between protein intake and cognition may have been affected by retrospective recall bias. With approximately 40% of participants diagnosed with MCI, there is a potential for inaccurate self-reported protein intake history. Nevertheless, while MCI individuals have recent memory issues, their remote memory remains intact [57]. Therefore, it is unlikely that participants with MCI reported their protein intake inaccurately, as these self-reports are based mainly on long-standing lifestyle habits rather than short-term memory. Additionally, our results remained consistent after adjusting for clinical diagnosis (CN vs. MCI) as an extra variable in Models 1 and 2 (Tables 2 and 4, and 5). Fourth, given the smaller cell sizes when the sample is broken down by APOE4 status, these findings should be interpreted with caution. Further research with larger sample sizes is necessary to confirm these findings and fully understand the implications of APOE4 on the relationship between protein intake and cognitive function. Lastly, we did not employ an objective method to measure protein intake. Similar to previous studies that used self-reported methods like food frequency questionnaires (FFQs) [18, 19] and semi-quantitative FFQs [20] to assess protein intake, our approach also has inherent constraints.

To address this, some studies have used objective methods like controlled dietary intervention [58], blood or urinary biomarkers (e.g., serum albumin [59] and urinary or plasma amino acid [60]) and isotope tracer methods (e.g., indicator amino acid oxidation [61, 62]) However, these methods have not been sufficiently validated in accurately reflecting dietary protein intake. Future research should combine self-reported dietary assessments with more precise objective methods to better validate the link between protein intake and cognitive function. Moreover, we did not differentiate protein intake by types, plant or animal sources, and processing methods. Given the ongoing debate over the impact of protein types (plant vs. animal sources) [63] and processing methods [64] on health outcomes, a more granular analysis in future studies is warranted.

Conclusions

Our study results demonstrated that high protein intake is associated with better episodic memory among older adults without dementia. Furthermore, the findings highlight the significant role of APOE4 status in moderating the relationship between protein consumption and episodic memory. These results suggest that dietary interventions focusing on protein intake could be beneficial for cognitive health, particularly in individuals with a genetic predisposition to AD.

Abbreviations

AD	Alzheimer's disease
APOE4	Apolipoprotein ε4
GLAD	General Lifestyle and Alzheimer's disease study
CN	Cognitively normal
MCI	Mild cognitive impairment
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
TS	Total score
VRS	Vascular risk score
GDS	Geriatric Depression Scale
MCL	Minimum cost of living
PASE	Physical Activity Scale for the Elderly
MNA	Mini Nutritional Assessment
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
VIF	Variance inflation factor
BDNF	Brain-derived neurotrophic factor

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Author contributions

M.K., B.C.L., Y.M.C., G-H.S., S.G.K., H.S.K., J.H., D.Y. and J.W.K. were responsible for the study concept and design. M.K. and B.C.L. performed the investigation and formal analysis. Y.M.C., G-H.S., S.G.K., H.S.K., J.H., and D.Y. contributed to the methodology, formal analysis, and writing of the original draft. M.K. and B.C.L. drafted the paper. J.W.K. handled conceptualization, funding acquisition, methodology, formal analysis, writing of the original draft, and supervision. All authors reviewed and critically revised the draft manuscript. Authors approved the final manuscript.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study protocol was approved by the Institutional Review Board of Hallym University Dongtan Sacred Heart Hospital and the study was conducted in accordance with the Declaration of Helsinki. All participants provided informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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