The impact of Alzheimer's disease and diabetes mellitus on the relation between plasma irisin and cognitive function

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ABSTRACT

The impact of Alzheimer's disease and diabetes mellitus on the relation between plasma irisin and cognitive function

Background and objective: Physical exercise is known to be a potential preventive measure for Alzheimer's Disease (AD) and diabetes mellitus (DM). Irisin, one of the key messengers in the brain-muscle axis, enhances cognitive and metabolic function. However, the relationship between blood levels of irisin and cognitive function remains unclear in the context of AD and DM. This study investigated the cross-sectional relationship of plasma irisin with cognitive function under various conditions of AD and DM, focusing on the mediating role of hippocampal volume. Additionally, this study also longitudinally explored whether plasma irisin predicts cognitive decline.

Methods: This study included 107 participants who were 50 years old or older. They were categorized based on cognitive status into three groups: cognitively normal (CN, n = 23), mild cognitive impairment (MCI, n = 49), and AD (n = 35). Participants were also categorized based on metabolic status into non-DM (n = 75) and DM (n = 32) groups. Blood for plasma irisin assay was collected after overnight fasting. Participants underwent MRI scans with analysis focusing on following regions of interest linked to AD and physical exercise: hippocampus, white matter hyperintensity, anterior cingulate gyrus, superior temporal gyrus, and inferior frontal gyrus. Cognitive function was annually assessed for mean follow-up period of 42.3 months. Double moderation analyses were applied to explore the impact of AD and DM on the relationship between plasma irisin and cognition, while hippocampal volume's mediating role was additionally tested in moderated mediation analyses. Linear mixed-effect models assessed the predictive value of plasma irisin.

Results: Double moderation analysis indicated that AD, rather than DM, significantly altered the association between plasma irisin levels and cognition or brain structures. In detail, increased plasma irisin levels were associated with better cognitive performance exclusively in participants without AD (CN plus MCI); However, this association was not observed in those with AD, where elevated plasma irisin levels were associated with reduced brain volumes. Moderated mediation models presented that, under the mediation of hippocampal volume, increased plasma irisin levels were associated with poorer cognitive function only in the presence of AD. Linear mixed-effect models revealed that baseline plasma irisin levels were not associated with changes in cognitive function. Baseline AD and DM statuses also did not significantly moderate the association between plasma irisin and longitudinal changes in cognitive function.

Conclusion: The results from cross-sectional analyses imply that the positive impact of plasma irisin on cognition diminishes in AD, supporting the idea of irisin playing a compensatory role during neurodegeneration and introducing the concept of irisin resistance. This possible concept of irisin resistance may be mediated by hippocampal volume. Meanwhile, longitudinal analyses

indicate that plasma irisin may not be appropriate for the prognostic biomarker for cognitive decline, irrespective of AD or DM statuses.

Key words : irisin, cognitive function, Alzheimer's disease, diabetes mellitus, hippocampus

ABBREVIATIONS

APOE: apolipoprotein E

BMI: body mass index

CDR-SB: clinical dementia rating - sum of boxes

CERAD-K: the Korean version of consortium to establish a registry for Alzheimer's disease

CN: cognitively normal

DM: diabetes mellitus

FDR: false discovery rate

HbA1c: glycosylated hemoglobin

HOMA-IR: homeostatic model assessment for insulin resistance

IFG: inferior frontal gyrus

MCI: mild cognitive impairment

MMSE: mini-mental status examination

MRI: magnetic resonance imaging

ROI: region of interest

SGDS: short form of geriatric depression scale

STG: superior temporal gyrus

TIV: total intracranial volume

WMH: white matter hyperintensity

1. INTRODUCTION

1.1. Alzheimer's disease (AD): importance of a lifelong approach

Dementia due to Alzheimer's disease (AD), the predominant type of neurodegenerative disease, ranks as the seventh most common cause of death worldwide and in Korea. [1,2] Mortality due to AD in Korea drastically increased from 4.1 per 100,000 in 2010 to 14.7 per 100,000 in 2020. [1] Global cost of dementia reached 972.3 billion USD in 2019, marking a 62% increase from 2010. [3] AD is characterized by the early pathologic process, such as deposition of amyloid-β (Aβ), decades before symptoms arise. [4,5] The insidious onset of AD underscores the importance of primary prevention and slowing disease progression. [6] The Lancet Commission reported that about 40% of dementia could be theoretically reduced by eliminating risks in mid and later life, such as physical inactivity or diabetes mellitus (DM). [6] Despite the development of disease-modifying anti-Aβ antibodies for AD, primary prevention of AD remains crucial due to the limitations in applying these treatments. [7]

1.2. Physical exercise: possible option for prevention of AD

Physical exercise, as evidenced by longitudinal cohort and controlled trial studies, is a possible option for preventing the progression of AD. [8-10] A recent population-based study confirmed that sedentary behavior measured by an accelerometer was significantly associated with subsequent risk of dementia. [11] The World Health Organization also strongly recommends physical activity to minimize the dementia risk. [12] Physical activity is associated with low amyloid burden in cognitively unimpaired individuals. [9] Previous randomized trials showed that individuals with aerobic exercise demonstrated increased volume in age-related brain regions[13] and better performance in cognitive tasks. [14]

These positive outcomes from physical exercise may result from improved cardiovascular and metabolic functions that are stimulated by exercise. Exercise and physical activity can ameliorate the risk of diabetes mellitus (DM), improve cardiovascular fitness, and mitigate obesity, [15] all of which are strongly associated with neurodegeneration

and dementia. [6] Meanwhile, mounting evidence indicates that physical exercise can directly affect the brain through the brain-muscle axis by myokines, molecules delivering signals from skeletal muscle. [16] For example, physical exercise stimulates brain-derived neurotrophic factor (BDNF) release, which is neurotrophic and neuroprotective. [17,18]

1.3. Irisin as a key molecule involving brain-muscle crosstalk

Irisin, a type of myokine, is produced when engaging in physical exercise by the cleavage of fibronectin type III domain-containing protein 5 (FNDC5). [19] Irisin level in the blood is elevated after both short-term bursts and prolonged periods of physical exercise. [20,21] Initially, the beneficial effect of irisin has been identified in enhancing glucose metabolism, promoting thermogenesis and converting white fat to brown fat. [19,22,23] Beyond glucose homeostasis, irisin also plays a crucial role in brain health. [16,24] It promotes the expression of neuroprotective genes like BDNF in hippocampus, thereby improving cognitive function. [25,26] In an AD mice model study, the peripheral irisin overexpression rescued cognitive impairment. [26] A recent study proved that irisin cleared Aβ pathology in AD's 3D cell culture model. [27] In humans, cerebrospinal fluid (CSF) irisin levels were decreased in AD patients. [26] Increased CSF irisin levels were associated with superior cognitive function and attenuated Aβ deposition. [28]

1.4. Relationship between blood irisin and cognition: need to consider the context of AD and DM

Although irisin is a circulating factor, and its concentration in CSF has been shown to correlate positively with cognitive function, [28] the relationship between blood irisin and cognition is not yet well understood. Prior studies of middle or older-aged individuals without dementia reported that increased blood irisin levels were associated with greater memory function. [29,30] Individuals with vascular dementia had lower blood irisin levels compared to those with normal cognition. [31] However, other studies have shown contradictory findings; blood irisin levels were similar between AD patients and individuals without AD. [26,32] Another prior study presented even higher plasma irisin levels in the MCI group than in controls. [33] Such mixed results indicate that the effect of peripheral circulating

irisin on the brain can be altered by AD status. It is possibly due to AD pathology which could interfere with the neuron's ability to respond to irisin.

As mentioned above, irisin also has a significant role in glucose metabolism. [23] Increased serum irisin was associated with fasting glucose and insulin resistance, [34] which are closely linked to neurodegeneration and cognitive decline. [35] Therefore, it is conceivable that diabetic status may also influence the association between circulating blood irisin and cognitive function.

1.5. Hippocampus: a potential mediator between circulating irisin and cognition

The hippocampus seems to be the key region of the brain where irisin has a role. A previous study with mice presented exercise-induced hippocampal BDNF elevation via peripheral FNDC5/irisin. [25] Another mouse model study reported that peripheral administration of FNDC5 increased the irisin level in the hippocampus, preventing memory impairment provoked by amyloid pathology. [26] This study group further revealed that peripheral irisin administration activated the protective signaling pathway in hippocampal neurons and prevented oxidative stress induced by Aβ. [36] However, the role of the hippocampus in the relationship between peripheral irisin and cognition in humans has much to be elucidated. Moreover, given the previous study that irisin administration increased BDNF in DM rats, [37] the relationship between irisin and cognition in the context of DM needs to be explored in the human observational study.

1.6. Irisin as a blood-based prognostic biomarker and the impact of AD and DM

Recently, several blood biomarkers were identified for predicting the progression of AD. [38] These biomarkers are advantageous compared to conventional CSF or neuroimaging biomarkers due to their non-invasiveness and cost-effectiveness. Given that irisin is a circulating protein that influences brain health, the prognostic potential of blood irisin for

cognitive decline warrants investigation. Moreover, the influence of AD and DM on the prognostic capacity of blood irisin remains to be fully elucidated.

1.7. Aims and hypothesis of this study

This study evaluated the association between plasma irisin and cognition, particularly considering the impact of AD and DM statuses. Participants were categorized based on their cognitive status into three groups: cognitively normal (CN), mild cognitive impairment (MCI), and AD. They were also classified by their diabetic condition into non-DM and DM groups. This study hypothesized that the association between irisin and cognition is attenuated when AD or DM exists. To validate the association between plasma irisin and cognition, magnetic resonance imaging (MRI) measured brain structural volumes. As a part of this study project, the results of the analyses mentioned above have been partially published. [39] Additionally, this study explored whether the hippocampus, a key brain region with significant irisin presence, [25,40] mediates the association between plasma irisin levels and cognitive function. This study also examined how this association is influenced by AD and DM statuses. Lastly, this study tested whether plasma irisin has predictive potential for cognitive decline in the context of AD and DM.

2. MATERIALS AND METHODS

2.1. Study participants

Enrollment and selection of this study sample are also described elsewhere. [39] This study enrolled participants from a university-affiliated general hospital and local dementia centers after confirming eligibility. Inclusion criteria were age 50 or above, literacy, and adequate hearing and vision to complete neuropsychological tests. The study excluded those with a history of schizophrenia or bipolar disorder, major neurologic diseases (including Parkinson's disease, major cerebrovascular disease, brain tumor, and epilepsy), or any medical conditions that could impact cognitive functions. Of the 115 participants, eight were excluded for the following reasons: one participant for clinical dementia rating (CDR) > 2, three for incomplete cognitive assessments, two for missing plasma irisin levels, one for the absence of an MRI scan, and one for unmeasured glycated hemoglobin A1c (HbA1c) level. Finally, 107 participants were analyzed in this study. Additionally, nine participants were excluded from certain analyses due to unavailable data on depression severity (four participants) or their history of smoking and alcohol consumption (five participants). Two board-certified specialists for old-age psychiatry determined the cognitive status (CN, MCI, or AD) using clinical interviews, neuroimaging, neuropsychological tests, and laboratory data. Cognitive function was assessed by the mini-mental state examination (MMSE) [41] and the Korean version of the consortium to establish a registry for Alzheimer's disease (CERAD-K). [42] Probable AD dementia based on the criteria of the National Institute on Aging and the Alzheimer's Association[43] was categorized as AD, and MCI was assessed according to Petersen's criteria. [44]

Participants were also grouped based on their metabolic conditions into DM (n = 32) and non-DM (n = 75). A board-certified endocrinologist diagnosed DM according to the American Diabetes Association's guidelines. [45] The criteria for a DM diagnosis included one or more of the following: fasting glucose level of 126 mg/dL or higher after fasting 8 hours, HbA1c of 6.5% or greater, glucose levels of 200 mg/dL or above after a 2-hour oral glucose tolerance test, or use of anti-diabetic medications.

Informed consent was obtained from all participants prior to enrollment, in accordance with the approval protocols of the institutional review board.

2.2. Procedure of blood drawing and plasma irisin assay

Venous blood was collected during the morning hours, specifically around 10 a.m., from the antecubital fossa of participants who had fasted overnight. The samples were promptly transported to the laboratory for further processing. Simultaneously, blood was thawed in heparinized tubes, which were centrifuged for 15 minutes at 2000 × g. This process facilitated the separation of plasma, which was subsequently apportioned into aliquots of 0.7 cc within multiple Eppendorf tubes and immediately frozen at −80 °C until the analysis. Alongside, apolipoprotein E ε4 (APOE4) genotyping was conducted. Apolipoprotein E genotyping was performed in accordance with the protocol detailed by Hixson and Vernier. [46] Plasma irisin was quantified by an enzyme-linked immunosorbent assay (ELISA) assay, adhering to the protocol provided by the manufacturer (Irisin ELISA Kit AG-45A-0046YEK-KI01; AdipoGen, Liestal, Switzerland).

2.3. Assessment of outcome variables

2.3.1. Cognitive function

As previously described, [39] participants in this study underwent comprehensive cognitive assessment using the CERAD-K neuropsychological battery, which includes the following: Verbal Fluency, Modified Boston Naming Test, MMSE, Word List Learning, Constructional Praxis, Word List Recall, Word List Recognition, Constructional Praxis Recall, Trail Making Test A (TMT-A), and Trail Making Test B (TMT-B). [42] Adding the results from Verbal Fluency, Modified Boston Naming Test, Word List Learning, Constructional Praxis, Word List Recall, Word List Recognition derived the total CERAD-K score, which ranges from 0 to 100. All the scores were standardized to Z-scores using established CERAD-K norms, which account for age, sex, and education level. [47,48] Z-scores of TMT-A and TMT-B were inverted (multiplied by -1) to align with other tests, making higher scores indicate better performance. Following a previous result of factor analysis, [42] five cognitive domains were categorized as follows: memory as mean of Word List Recall, Word List Recognition, and Word List Learning; language as mean of Verbal Fluency and Modified Boston Naming Test; visuospatial function as mean of Constructional Praxis and Constructional Praxis Recall; attention as TMTA; and executive

function as TMT-B. Overall cognitive function was represented by the Z-scores of the total CERAD-K and MMSE.

The CDR–sum of boxes (CDR-SB) was used to evaluate the severity and functional impairment of dementia, as it is widely adopted as a primary outcome measure in recent dementia studies. [49]

2.3.2. Brain structure: MRI scan, preprocessing, and analysis

A 3.0T Philips Intera Achieva scanner (Philips Medical Systems, Best, the Netherlands) was used to acquire brain MRI images. Gray matter volume was quantified using 3D T1-weighted images obtained with a repetition time of 9,300 ms and an echo time of 4.6 ms. These images underwent automated processing utilizing the Computational Anatomical Toolbox 12 within Statistical Parametric Mapping software 12 on the MATLAB 2014a. The T1-weighted images were first enhanced with a spatial-adaptive non-local means denoising filter and then partitioned into gray matter, white matter, and CSF compartments using an adaptive maximum posterior segmentation approach. Then partial volume estimation delineated two mixed tissue classes, such as gray matter-white matter and gray matter-CSF, to improve segmentation accuracy. The gray matter images were then spatially normalized, ensuring the conservation of the total GM signal within the normalized partitions.

A 3D T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence with repetition time of 11,000 ms and echo time of 346 ms was employed to assess white matter hyperintensity (WMH). WMH lesions were identified using an established automated algorithm. [50,51] This study made two adjustments compared to earlier work: firstly, the application of an optimal threshold value of 70 (up from the original 65) for better specificity; and secondly, excluding diffusion-weighted imaging due to the exclusion of participants with acute ischemic infarctions. The WMH volumes were measured within lobar ROIs in each participant's native space. [52]

Regions of interest (ROIs) in this study were hippocampus, WMH, anterior cingulate gyrus (ACG), superior temporal gyrus (STG), and inferior frontal gyrus (IFG). ROIs were determined using the Neuromorphometric atlas (http://www.neuromorphometric.com). Hippocampus and WMH are core regions for the prediction of AD progression. [53,54] Moreover, irisin is predominantly found in the hippocampus, which is regarded as the main target region of irisin. [25,26] ACG, STG, and IFG are the main areas where the effect of physical exercise can be seen. [13] The bilateral

ROI volumes were also adjusted for the total intracranial volume (TIV) expressed as per mille to account for variations in head size.

2.4. Assessment of other variables

Each participant was evaluated for depression severity using the short form of the geriatric depression scale (SGDS). [55] The SGDS is composed of 15 yes-or-no questions; higher total scores, up to a maximum of 15, indicate more severe depression symptoms. Histories of hypertension, smoking, and alcohol abuse were obtained through self-reports or reviews of medical records. The body mass index (BMI) of each participant was measured to assess the degree of obesity. To evaluate diabetic conditions, HbA1c and homeostatic model assessment for insulin resistance (HOMA-IR) were measured. Participants reported the frequency of their physical activity lasting more than 30 minutes, with the frequency recorded in days per week, ranging from 0 to 7.

2.5. Statistical analysis

2.5.1. Comparison between groups

Demographic and clinical characteristics between three cognitive groups were compared using analysis of variance (ANOVA) and chi-square test or Fisher's exact test for continuous variables and categorical variables, respectively. Following ANOVA, the Bonferroni method for post hoc analyses was employed. To compare participants with and without DM, Student's t-test and chi-square test or Fisher's exact test were used.

2.5.2. Association between plasma irisin and outcome variables

Multiple linear regression models evaluated associations between plasma irisin levels and cognitive function or volumes of ROIs after stratifying participants by cognitive (CN, MCI, or AD) or diabetic status (non-DM or DM). Two linear regression models for each outcome variable were conducted. Model 1 adjusted for age, sex, and education level (years), while model 2 also accounted for SGDS, hypertension, smoking history, alcohol abuse history, BMI, HbA1c, APOE4, and exercise frequency. Priorly, plasma irisin levels were natural log-transformed due to non-normal and skewed distribution.

2.5.3. Moderation effect of AD and DM (double moderation analysis)

Double moderation models were applied to explore how AD and DM influence the relationship between plasma irisin and outcome variables (cognitive test scores, CDR-SB, and volumes of ROIs) [39] (Figure 1). In this model, the interaction terms plasma irisin level × presence or absence of AD (and DM) were put into multiple regression analyses. Plasma irisin level served as the explanatory variable, while cognitive function and volume of ROIs were outcome variables. The analysis controlled for the same covariates in multiple linear regression model 2. If the interaction term was significant, indicating the noticeable moderation effect of AD or DM, associations between plasma irisin and outcome variables were then evaluated under various AD and DM conditions (i.e., non-AD/non-DM, AD/non-DM, non-AD/DM, and AD/DM).

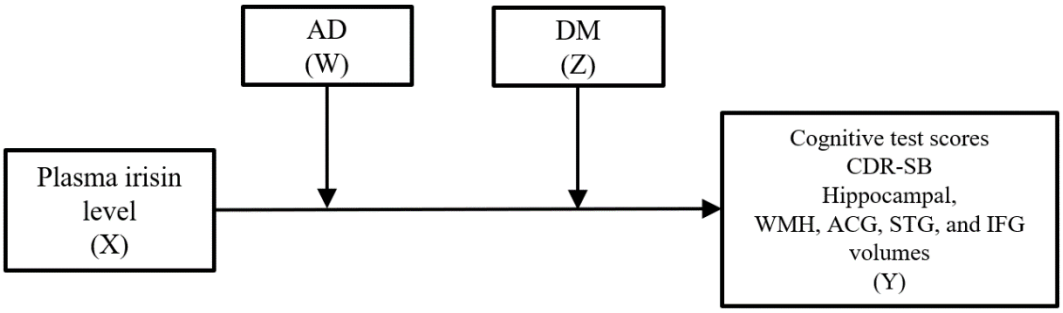

Figure 1. Double moderation model. [39] The outcome variables were cognition, CDR-SB, and ROI volume (Y). The explanatory variable was plasma irisin level (X). Presence or absence of AD and DM was the moderator variable (W and Z).
Double moderation models were assessed using PROCESS Macro model number 2. [56]
Abbreviations are explained on page viii.

2.5.4. Moderation effect of AD and DM in hippocampal mediation between plasma irisin and cognitive function (moderated mediation analysis)

This analysis investigated the moderation effect of AD and DM on the mediation model, where hippocampal volume serves as a mediator between plasma irisin and cognition (Figure 2). The sum of bilateral hippocampal volumes was regarded as the total hippocampal volume. Plasma irisin level served as the explanatory variable, and the outcome variables were cognitive function scores and CDR-SB. Covariates in the analysis were the same as those in the linear regression models. The analysis calculated the indirect effect of plasma irisin on cognition, mediated by hippocampal volume, across various AD and DM statuses.

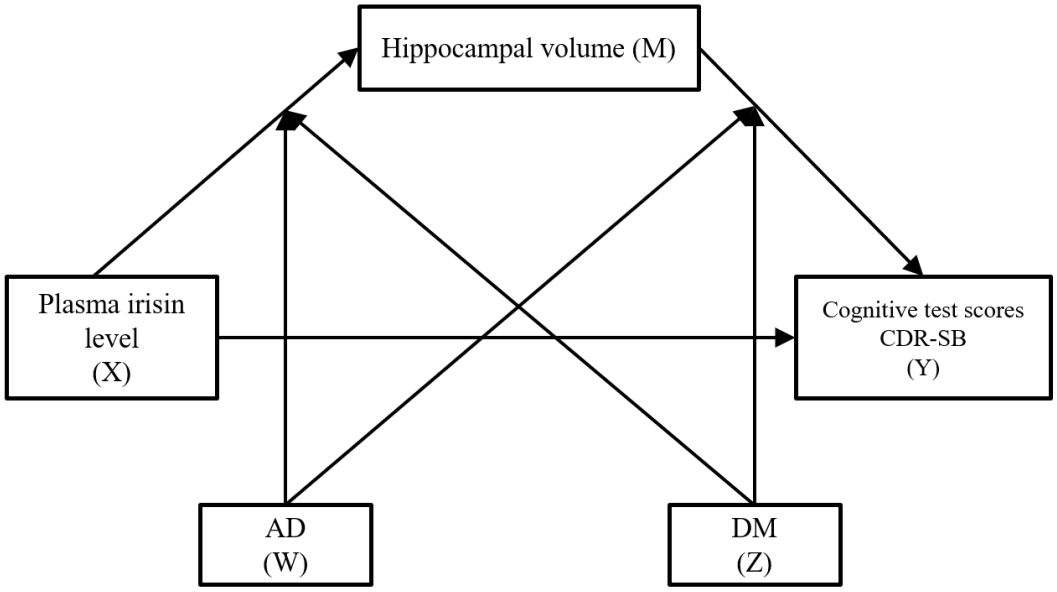

Figure 2. Moderated mediation model. The outcome variables were cognition and CDR-SB (Y). Plasma irisin level was the explanatory variable (X). Hippocampal volume was the mediator (M), and the presence or absence of AD and DM were the moderators (W and Z) of the indirect effect of irisin via hippocampal volume.
Moderated mediation models were assessed using PROCESS Macro model number 75. [56]

2.5.5. Association between plasma irisin and longitudinal cognitive function over time

Linear mixed-effect models were used to evaluate the predictive potential of plasma irisin for changes in cognitive function. Dependent variables included cognitive function scores and CDR-SB, while the explanatory variable was the interaction term plasma irisin × time (months). Random effects were intercept and slope. Fixed effects included plasma irisin level, time since baseline (months), and the interaction between plasma irisin level and time since baseline (months). Covariates were same as those in the linear regression models.

Additional linear mixed-effect models were used to assess the influence of AD

and DM on the predictive potential of plasma irisin for cognitive decline. Fixed effects in the model encompassed plasma irisin level, time since baseline, AD or DM, and relevant interaction terms, including plasma irisin × time × AD (or DM). Other variables were the same as those in prior linear mixed-effect models. For missing data, listwise deletion was used.

2.5.6. Software computing statistics

Statistical analyses were conducted using R, version 4.3.1 (R Foundation for Statistical Computing), with a significance level set at a p value of 0.05 or within a 95% confidence interval. False discovery rate (FDR) correction using Benjamini-Hochberg method was applied to adjust p values accounting for multiple outcomes. Double moderation models (Figure 1) and moderated mediation models (Figure 2) were fitted by PROCESS macro for R by Hayes. [56] The lme4 package, version 1.1–33, was used to fit linear mixed-effect models.

3. RESULTS

3.1. Characteristics of participants according to cognitive and diabetic statuses

Clinical and demographic sample characteristics are summarized in Table 1. Participants diagnosed with CN exhibited higher education levels, fewer depressive symptoms, and shorter follow-up periods compared to those with MCI and AD. Notably, plasma irisin levels were increased in participants with CN relative to those with MCI and AD. HbA1c and HOMA-IR showed no significant difference across the different cognitive statuses. Compared to participants without DM, those with DM had a lower proportion of females, higher education levels, were more likely to be smokers, and engaged in exercise more frequently. Plasma irisin levels were similar between participants with and without DM.

Table 1. Demographic and characteristics of participants[39]

Characteristic	Total (n = 107)	CN (n = 23)	MCI (n = 49)	AD (n = 35)	p value	Post hoc	Non-DM (n = 75)	DM (n = 32)	p value
Age (years)	71.3 (4.80)	71.0 (5.90)	71.4 (4.73)	71.3 (4.20)	0.951		71.5 (4.73)	70.7 (5.00)	0.440
Sex (n, % of female)	78 (72.9%)	17 (73.9%)	36 (73.5%)	25 (71.4%)	0.971		64 (85.3%)	14 (43.8%)	<0.001
Education (years)	8.18 (4.98)	11.3 (4.84)	8.12 (4.45)	6.20 (4.86)	<0.001	CN> MCI, AD	7.55 (4.85)	9.66 (5.04)	0.044
SGDS*	6.48 (4.24)	3.32 (3.23)	6.06 (3.80)	8.77 (4.08)	<0.001	CN< MCI <AD	6.82 (4.00)	5.72 (4.69)	0.225
Hypertension	50 (46.7%)	9 (39.1%)	22 (44.9%)	19 (54.3%)	0.496		30 (40.0%)	20 (62.5%)	0.054
Ever smoker*	12 (11.2%)	1 (4.3%)	7 (14.3%)	4 (11.4%)	0.491		5 (6.7%)	7 (21.9%)	0.045
Alcohol abuse history*	5 (4.7%)	1 (4.3%)	3 (6.1%)	1 (2.9%)	0.849		2 (2.7%)	3 (9.4%)	0.300
BMI (kg/m^2)	24.3 (2.65)	23.9 (2.18)	24.4 (2.93)	24.4 (2.57)	0.693		24.1 (2.46)	24.8 (3.03)	0.159
DM	32 (29.9%)	5 (21.7%)	15 (30.6%)	12 (34.3%)	0.588				
HbA1c (%)	6.38 (0.99)	6.17 (0.89)	6.37 (0.91)	6.54 (1.15)	0.378		5.88 (0.33)	7.56 (1.01)	<0.001
HOMA-IR (units)*	2.51 (1.83)	2.04 (1.11)	2.51 (1.91)	2.82 (2.08)	0.291		2.01 (1.00)	3.66 (2.66)	0.002
APOE4 count					0.022	MCI <AD			
0	83 (77.6%)	19 (82.6%)	43 (87.8%)	21 (60.0%)			58 (77.3%)	25 (78.1%)	0.640
1	22 (20.6%)	4 (17.4%)	6 (12.2%)	12 (34.3%)			15 (20.0%)	7 (21.9%)	
2	2 (1.9%)	0 (0%)	0 (0%)	2 (5.7%)			2 (2.7%)	0 (0%)	
Exercise frequency (per week)*	2.20 (2.74)	3.13 (2.99)	1.96 (2.74)	1.88 (2.47)	0.176		1.63 (2.45)	3.57 (2.94)	<0.001
Plasma irisin level (ng/mL)	2.49 (1.75)	3.33 (2.79)	2.34 (1.30)	2.16 (1.24)	0.031	CN> MCI, AD	2.58 (1.87)	2.30 (1.46)	0.461
Follow-up months	42.3 (47.0)	19.5 (39.1)	50.9 (51.1)	45.1 (41.7)	0.026	CN< MCI	39.9 (47.0)	47.8 (47.4)	0.431

Data are presented as mean ± standard deviation for continuous variables and n (%) for categorical variables.
Post-hoc comparisons were corrected for FDR using Benjamini-Hochberg procedure.
*Some data were missed (n = 103 for SGDS, n = 102 for ever smoking, alcohol abuse history, and exercise frequency, and n = 106 for HOMA-IR).
Abbreviations are explained on page viii.

3.2. Cognition and ROI volume according to cognitive and diabetic statuses

Table 2 displays the cognitive function and volumes of ROIs. The AD group demonstrated significantly lower performance across all five cognitive domains. Participants with AD had smaller left hippocampal volumes compared to those with CN. There were no significant differences in cognitive function and ROI volumes between participants with and without DM, except for right IFG volumes, which were smaller in those without DM.

Table 2. Cognition and brain volumes[39]

Outcome	Total (n = 107)	CN (n = 23)	MCI (n = 49)	AD (n = 35)	p value	Post hoc	Non-DM (n = 75)	DM (n = 32)	p value
MMSE	22.6 (4.87)	27.5 (1.95)	23.7 (3.14)	18.0 (4.27)	< 0.001	CN>MCI>AD	22.8 (4.86)	22.3 (4.96)	0.647
CDR-SB	2.37 (1.94)	0.435 (0.679)	2.03 (0.780)	4.11 (2.17)	< 0.001	CN<MCI<AD	2.23 (1.82)	2.69 (2.18)	0.269
Memory	24.0 (9.04)	34.4 (6.39)	24.1 (6.47)	17.0 (6.75)	< 0.001	CN>MCI>AD	24.6 (9.21)	22.7 (8.64)	0.329
Language	21.6 (7.13)	27.5 (5.54)	22.5 (5.73)	16.3 (6.25)	< 0.001	CN>MCI>AD	21.8 (7.13)	21.0 (7.22)	0.577
Visuospatial function	13.2 (4.38)	16.8 (2.79)	13.6 (4.10)	10.4 (3.73)	< 0.001	CN>MCI>AD	13.1 (4.24)	13.6 (4.72)	0.624
Attention*	112 (92.8)	58.6 (34.4)	92.3 (65.7)	174 (116)	< 0.001	CN<MCI, AD	120 (100)	92.1 (70.9)	0.101
Executive function*	254 (81.6)	199 (89.3)	253 (82.7)	292 (49.1)	< 0.001	CN<MCI<AD	258 (80.7)	244 (84.1)	0.411
Total CERAD-K‡	54.6 (15.8)	72.0 (11.3)	55.7 (10.8)	41.5 (12.0)	< 0.001	CN>MCI>AD	55.3 (16.0)	52.9 (15.2)	0.476
Hippocampus/TIV, right (‰)	2.23 (0.45)	2.37 (0.39)	2.26 (0.41)	2.09 (0.52)	0.055		2.23 (0.39)	2.23 (0.57)	0.997
Hippocampus/TIV, left (‰)	1.99 (0.38)	2.13 (0.35)	2.02 (0.32)	1.86 (0.44)	0.021	CN>AD	1.97 (0.36)	2.03 (0.41)	0.501
WMH/TIV (‰)	3.95 (4.40)	2.49 (2.17)	4.24 (4.51)	4.48 (5.15)	0.211		3.91 (4.13)	4.05 (5.08)	0.881
ACG/TIV, right (‰)	2.46 (0.54)	2.41 (0.59)	2.57 (0.56)	2.34 (0.45)	0.147		2.45 (0.53)	2.50 (0.55)	0.654
ACG/TIV, left (‰)	3.19 (0.56)	3.22 (0.54)	3.21 (0.59)	3.14 (0.54)	0.851		3.14 (0.52)	3.31 (0.63)	0.146
STG/TIV, right (‰)	4.23 (0.73)	4.22 (0.77)	4.27 (0.64)	4.16 (0.85)	0.810		4.16 (0.73)	4.38 (0.73)	0.168
STG/TIV, left (‰)	3.88 (0.62)	3.95 (0.56)	3.90 (0.57)	3.82 (0.74)	0.716		3.82 (0.54)	4.04 (0.77)	0.138
IFG/TIV, right (‰)	2.03 (0.37)	2.04 (0.34)	2.04 (0.38)	2.02 (0.38)	0.948		1.99 (0.34)	2.14 (0.41)	0.046
IFG/TIV, left (‰)	1.98 (0.37)	2.02 (0.29)	2.02 (0.38)	1.89 (0.40)	0.220		1.96 (0.36)	2.01 (0.40)	0.488

Data are presented as mean ± standard deviation.
Post-hoc comparisons were corrected for FDR using Benjamini-Hochberg procedure.
*Unlike other cognitive domains, higher attention and executive function scores indicate poorer performance.
Abbreviations are explained on page viii.

3.3. Association between plasma irisin and cognition across clinical diagnosis of cognitive statuses

Multiple linear regression models for the cross-sectional association between plasma irisin and cognitive function are summarized in Table 3. In participants with CN and MCI, no notable association between plasma irisin and any cognitive domain or CDR-SB was observed (Table 3, second and third panel from the left). However, in patients without AD, encompassing both CN and MCI groups, plasma irisin levels showed significant positive association with memory and total CERAD-K scores in model 1, and executive function and total CERAD-K scores in model 2 (Table 3, leftmost panel). In contrast, in the AD group, plasma irisin levels were inversely associated with attention scores in model 2 (Table 3, rightmost panel). After adjusting p values using Benjamini-Hochberg procedure, none of the models retained statistical significance.

Table 3. Association between plasma irisin and cognition: stratified by clinical diagnosis of cognitive status

	Non-AD (n = 65)			CN (n = 19)			MCI (n = 46)			AD (n = 33)		
Outcome	Standardized beta	p value	Adjusted p value	Standardized beta	p value	Adjusted p value	Standardized beta	p value	Adjusted p value	Standardized beta	p value	Adjusted p value
MMSE												
Model 1	0.208	0.081	0.130	0.005	0.986	0.986	0.153	0.297	0.558	-0.203	0.274	0.556
Model 2	0.260	0.061	0.126	-0.357	0.404	0.715	0.051	0.775	0.775	-0.237	0.319	0.638
CDR-SB												
Model 1	-0.144	0.208	0.277	-0.034	0.880	0.986	0.008	0.957	0.957	0.216	0.258	0.556
Model 2	-0.127	0.318	0.364	-0.166	0.786	0.794	0.071	0.690	0.775	0.397	0.076	0.303
Memory												
Model 1	0.238	0.046	0.130	0.100	0.685	0.986	0.134	0.349	0.558	-0.140	0.456	0.558
Model 2	0.214	0.107	0.170	0.096	0.761	0.794	0.134	0.430	0.688	-0.025	0.912	0.912
Language												
Model 1	0.206	0.081	0.130	0.118	0.637	0.986	0.210	0.155	0.465	-0.132	0.486	0.556
Model 2	0.238	0.063	0.126	-0.400	0.447	0.715	0.163	0.318	0.660	-0.111	0.628	0.838
Visuospatial function												
Model 1	0.012	0.922	0.922	-0.221	0.358	0.954	0.009	0.951	0.957	-0.077	0.641	0.641
Model 2	-0.025	0.860	0.860	-0.437	0.357	0.715	-0.087	0.605	0.775	-0.132	0.521	0.834
Attention												
Model 1	-0.128	0.254	0.290	0.254	0.277	0.954	-0.247	0.069	0.465	-0.141	0.418	0.556
Model 2	-0.152	0.227	0.303	-0.375	0.444	0.715	-0.278	0.064	0.510	-0.416	**0.035**	0.276
Executive function												
Model 1	0.207	0.061	0.130	0.406	0.063	0.507	0.106	0.450	0.600	-0.275	0.149	0.556
Model 2	0.262	**0.018**	0.126	0.436	0.182	0.715	0.153	0.330	0.660	-0.300	0.181	0.483
Total CERAD-K												
Model 1	0.240	**0.043**	0.130	0.040	0.872	0.986	0.204	0.174	0.465	-0.143	0.447	0.556
Model 2	0.258	**0.046**	0.126	-0.113	0.794	0.794	0.180	0.304	0.660	-0.056	0.822	0.912

MMSE, memory, language, visuospatial function, attention, executive function, and total CERAD-K scores were standardized to Z-scores.

Covariates in model 1: age, sex, and education level (years).

Covariates in model 2: (in addition to model 1) SGDS, hypertension, smoking, alcohol abuse history, BMI, HbA1c, APOE4, and exercise frequency.
Bold signals represent statistical significance ($p < 0.05$).
P values were adjusted for FDR using Benjamini-Hochberg procedure.
Abbreviations are explained on page viii.

3.4. Association between plasma irisin and ROI volume across clinical diagnosis of cognitive statuses

The relationship between plasma irisin and brain volumes of ROIs was investigated (Table 4). In contrast to the findings that plasma irisin levels were positively correlated with cognitive function in participants without AD (Table 3), no significant associations were observed between plasma irisin levels and volumes of ROIs in these participants (Table 4, leftmost panel). However, a significant negative association between plasma irisin levels and ROI volumes was observed in participants with AD (model 1: left hippocampus and right STG; model 2: left hippocampus, bilateral STG, and left IFG; Table 4, rightmost panel). These findings suggest that the association between plasma irisin and both cognitive function and brain structure may be influenced by clinically diagnosed cognitive status.

Table 4. Association between plasma irisin and ROI volume: stratified by clinical diagnosis of cognitive status

	Non-AD (n = 65)			CN (n = 19)			MCI (n = 46)			AD (n = 33)		
Outcome	Standardized beta	p value	Adjusted p value	Standardized beta	p value	Adjusted p value	Standardized beta	p value	Adjusted p value	Standardized beta	p value	Adjusted p value
Hippocampus/TIV, right												
Model 1	-0.001	0.994	0.994	-0.066	0.776	0.816	-0.021	0.881	0.922	-0.432	**0.021**	0.064
Model 2	0.024	0.826	0.917	0.315	0.416	0.535	-0.053	0.708	0.945	-0.468	**0.034**	0.061
Hippocampus/TIV, left												
Model 1	-0.064	0.558	0.832	-0.182	0.437	0.787	-0.054	0.670	0.922	-0.502	**0.006**	**0.027**
Model 2	0.022	0.837	0.917	0.440	0.303	0.488	-0.018	0.885	0.945	-0.473	**0.012**	**0.035**
WMH/TIV												
Model 1	-0.079	0.523	0.832	-0.276	0.265	0.632	0.027	0.861	0.922	0.072	0.675	0.675
Model 2	-0.088	0.529	0.917	-0.895	0.118	0.377	0.052	0.778	0.945	0.108	0.634	0.634
ACG/TIV, right												
Model 1	-0.074	0.539	0.832	-0.056	0.816	0.816	-0.097	0.517	0.922	-0.210	0.266	0.299
Model 2	-0.074	0.529	0.917	0.464	0.263	0.488	-0.091	0.568	0.945	-0.158	0.452	0.508
ACG/TIV, left												
Model 1	-0.042	0.725	0.832	-0.073	0.767	0.816	-0.042	0.772	0.922	-0.275	0.132	0.198
Model 2	-0.040	0.745	0.917	0.461	0.325	0.488	-0.058	0.731	0.945	-0.292	0.120	0.155
STG/TIV, right												
Model 1	-0.117	0.318	0.832	-0.316	0.159	0.632	-0.074	0.593	0.922	-0.472	**0.005**	**0.027**
Model 2	-0.034	0.775	0.917	0.146	0.719	0.719	-0.011	0.945	0.945	-0.647	**< 0.001**	**0.001**
STG/TIV, left												
Model 1	-0.038	0.740	0.832	-0.298	0.164	0.632	0.018	0.898	0.922	-0.289	0.097	0.175
Model 2	0.012	0.917	0.917	-0.192	0.561	0.631	0.080	0.616	0.945	-0.464	**0.008**	**0.035**
IFG/TIV, right												
Model 1	-0.045	0.701	0.832	-0.067	0.780	0.816	-0.087	0.525	0.922	-0.258	0.170	0.219
Model 2	-0.018	0.881	0.917	0.752	0.065	0.377	-0.074	0.617	0.945	-0.314	0.076	0.114
IFG/TIV, left												
Model 1	0.094	0.433	0.832	0.272	0.281	0.632	0.014	0.922	0.922	-0.365	0.052	0.118
Model 2	0.129	0.315	0.917	0.880	0.126	0.377	0.105	0.513	0.945	-0.343	**0.020**	**0.045**

Covariates in model 1: age, sex, and education level (years).
Covariates in model 2: (in addition to model 1) SGDS, hypertension, smoking, alcohol abuse history, BMI, HbA1c, APOE4, and exercise frequency.
Bold signals represent statistical significance ($p < 0.05$).
P values were adjusted for FDR using Benjamini-Hochberg procedure.
Abbreviations are explained on page viii.

3.5. Association between plasma irisin and cognitive function across diabetic statuses

Table 5 presents the results of multiple linear regression models, stratified by diabetic statuses (non-DM and DM), with cognitive function and CDR-SB as outcome variables. For participants without DM, any significant association was not observed between plasma irisin levels and any cognitive domain or CDR-SB in either regression models 1 or 2 (Table 5, left panel). Similarly, among participants with DM, no significant associations were observed between plasma irisin levels and cognitive outcomes after adjusting p values, mirroring the results seen in those without DM (Table 5, right panel)

Table 5. Association between plasma irisin and cognition: stratified by DM status

Outcome	Non-DM (n = 68)			DM (n = 30)		
	Standardized beta	p value	Adjusted p value	Standardized beta	p value	Adjusted p value
MMSE						
Model 1	-0.017	0.879	0.879	0.413	**0.032**	0.106
Model 2	-0.030	0.808	0.924	0.268	0.282	0.517
CDR-SB						
Model 1	0.077	0.491	0.870	-0.352	0.066	0.106
Model 2	-0.030	0.808	0.921	0.268	0.282	0.767
Memory						
Model 1	0.104	0.355	0.870	0.316	0.085	0.113
Model 2	0.084	0.466	0.924	0.232	0.323	0.517
Language						
Model 1	0.031	0.788	0.879	0.366	0.057	0.106
Model 2	-0.016	0.890	0.924	0.442	0.083	0.333
Visuospatial function						
Model 1	-0.115	0.346	0.870	0.240	0.195	0.223
Model 2	-0.119	0.345	0.921	-0.021	0.930	0.930
Attention						
Model 1	-0.142	0.213	0.870	0.153	0.401	0.401
Model 2	-0.165	0.169	0.921	-0.038	0.857	0.930
Executive function						
Model 1	0.027	0.800	0.879	0.420	**0.021**	0.106
Model 2	-0.010	0.924	0.924	0.446	0.057	0.333
Total CERAD-K						
Model 1	0.068	0.544	0.870	0.388	**0.045**	0.106
Model 2	0.046	0.683	0.924	0.343	0.204	0.517

MMSE, memory, language, visuospatial function, attention, executive function, and total CERAD-K scores were converted to Z-scores.
Covariates in model 1: age, sex, and education level (years).
Covariates in model 2: (in addition to model 1) SGDS, hypertension, smoking, alcohol abuse history, BMI, HbA1c, APOE4, and exercise frequency.
Bold signals represent statistical significance ($p < 0.05$).
Adjusted p values were corrected for FDR using Benjamini-Hochberg procedure.
Abbreviations are explained on page viii.

3.6. Association between plasma irisin and ROI volume across diabetic statuses

Table 6 displays the results of multiple linear regression models in which volumes of ROIs were outcome variables. Among participants without DM, plasma irisin levels were inversely associated with right STG in regression model 1 after adjusting p values. This association lost significance in regression model 2 (Table 6, left panel). In contrast, no significant association was found between plasma irisin levels and the volumes of ROIs among participants with DM (Table 6, right panel).

Table 6. Association between plasma irisin and ROI volume: stratified by DM status

Outcome	Non-DM (n = 68)			DM (n = 30)		
	Standardized beta	p value	Adjusted p value	Standardized beta	p value	Adjusted p value
Hippocampus/TIV, right						
Model 1	-0.249	**0.022**	0.053	0.106	0.587	0.755
Model 2	-0.234	**0.038**	0.113	-0.391	0.066	0.594
Hippocampus/TIV, left						
Model 1	-0.256	**0.019**	0.053	0.028	0.887	0.887
Model 2	-0.215	0.062	0.140	-0.291	0.164	0.738
WMH/TIV						
Model 1	0.048	0.690	0.690	-0.181	0.338	0.721
Model 2	0.126	0.343	0.441	-0.289	0.276	0.827
ACG/TIV, right						
Model 1	-0.203	0.077	0.116	0.196	0.314	0.721
Model 2	-0.187	0.103	0.171	-0.125	0.489	0.857
ACG/TIV, left						
Model 1	-0.250	**0.024**	0.053	0.220	0.263	0.721
Model 2	-0.233	**0.037**	0.113	0.003	0.991	0.991
STG/TIV, right						
Model 1	-0.365	**0.002**	**0.014**	0.163	0.401	0.721
Model 2	-0.306	**0.014**	0.113	-0.072	0.762	0.857
STG/TIV, left						
Model 1	-0.234	**0.045**	0.080	0.134	0.487	0.730
Model 2	-0.198	0.114	0.171	-0.098	0.679	0.857
IFG/TIV, right						
Model 1	-0.159	0.168	0.215	0.056	0.779	0.876
Model 2	-0.090	0.471	0.471	-0.112	0.633	0.857
IFG/TIV, left						
Model 1	-0.093	0.425	0.478	0.179	0.366	0.721
Model 2	-0.088	0.454	0.471	-0.079	0.761	0.857

Covariates in model 1: age, sex, and education level (years).
Covariates in model 2: (in addition to model 1) SGDS, hypertension, smoking, alcohol abuse history, BMI, HbA1c, APOE4, and exercise frequency.
Bold signals represent statistical significance ($p < 0.05$).
Adjusted p values were corrected for FDR using Benjamini-Hochberg procedure.
Abbreviations are explained on page viii.

3.7. Impact of AD and DM on association between plasma irisin and cognition and ROI volume: double moderation analysis

After confirming that the association between plasma irisin levels and cognition or brain structure varied under different cognitive or diabetic conditions (Table 3 to 6), double moderation models were applied to assess the moderating effect of AD and DM, as illustrated in Figure 1. The results are summarized in Table 7. Notably, the presence of AD significantly moderated the association between plasma irisin and the following outcome variables: MMSE, CDR-SB, language, executive function, total CERAD-K, bilateral hippocampus, bilateral STG, and left IFG (Table 7, left panel). In contrast, the presence of DM did not significantly influence the association between plasma irisin levels and any of the measured outcomes (Table 7, right panel). These findings indicate that varying associations observed under different cognitive and diabetic conditions, as shown in Table 3 to 6, are attributable to the moderating effect of AD, rather than DM.

Table 7. Impact of AD and DM on the association between plasma irisin and cognition or ROI volume

Outcome	Moderating effect of AD					Moderating effect of DM				
	Unstandardized beta	SE	t	p-value for irisin × AD	Adjusted p value	Unstandardized beta	SE	t	p-value for irisin × DM	Adjusted p value
MMSE	-1.287	0.520	-2.477	**0.015**	**0.036**	0.808	0.498	1.621	0.109	0.402
CDR-SB	1.510	0.650	2.325	**0.023**	**0.036**	-0.903	0.623	-1.450	0.151	0.402
Memory	-0.910	0.499	-1.823	0.072	0.082	-0.056	0.479	-0.116	0.908	0.908
Language	-0.885	0.380	-2.331	**0.022**	**0.036**	0.430	0.364	1.182	0.241	0.481
Visuospatial function	-0.467	0.451	-1.035	0.304	0.304	0.258	0.433	0.595	0.553	0.672
Attention	-1.550	0.795	-1.950	0.055	0.073	0.725	0.762	0.952	0.344	0.550
Executive function	-1.531	0.523	-2.928	**0.004**	**0.035**	0.925	0.501	1.845	0.069	0.402
Total CERAD-K	-1.249	0.538	-2.323	**0.023**	**0.036**	0.281	0.516	0.544	0.588	0.672
Hippocampus/TIV, right	-0.607	0.172	-3.534	**0.001**	**0.002**	-0.115	0.165	-0.697	0.488	0.732
Hippocampus/TIV, left	-0.536	0.140	-3.833	**<0.001**	**0.001**	-0.104	0.134	-0.772	0.443	0.732
WMHL/TIV	2.767	2.151	1.286	0.202	0.227	-2.107	2.064	-1.021	0.311	0.732
ACG/TIV, right	-0.205	0.248	-0.827	0.411	0.411	0.166	0.238	0.701	0.486	0.732
ACG/TIV, left	-0.417	0.246	-1.696	0.094	0.141	0.171	0.236	0.724	0.471	0.732
STG/TIV, right	-1.138	0.303	-3.756	**<0.001**	**0.001**	0.289	0.291	0.996	0.322	0.732
STG/TIV, left	-0.732	0.276	-2.654	**0.010**	**0.022**	0.021	0.265	0.080	0.936	0.936
IFG/TIV, right	-0.257	0.165	-1.555	0.124	0.159	-0.065	0.159	-0.411	0.682	0.877
IFG/TIV, left	-0.406	0.167	-2.430	**0.017**	**0.031**	-0.016	0.160	-0.101	0.920	0.936

MMSE, memory, language, constructional ability, attention, executive function, and total CERAD-K scores were standardized to Z-scores.

Models were adjusted for age, sex, education level (years), SGDS, hypertension, smoking, alcohol abuse, BMI, HbA1c,

APOE4, and exercise frequency.
Bold signals represent statistical significance ($p < 0.05$).
Adjusted p values were corrected for FDR using Benjamini-Hochberg procedure.
Abbreviations are explained on page viii.

3.8. Conditional effect of plasma irisin under different cognitive or diabetic conditions: derived from double moderation models

The relationship between plasma irisin levels and cognition under various cognitive and diabetic conditions is visualized in Figure 3. MMSE, CDR-SB, language, executive function, and total CERAD-K were depicted in Figure 3 due to their moderated association with plasma irisin in the context of AD (Table 7). In the non-AD group, increased plasma irisin levels were associated with higher cognitive function (Figure 3A to 3E, magenta line). However, these associations were absent or even reversed in those with AD (Figure 3A to 3E, teal blue line). In contrast, DM did not significantly influence the relationship between plasma irisin levels and cognition. (Figure 3A to 3E, right and left panels).

Conditional association between plasma irisin and volumes of ROIs stratified AD/DM status are depicted in Figure 4. The volumes of bilateral hippocampus, bilateral STG, and left IFG are visualized in Figure 4, reflecting their significant associations with plasma irisin that were moderated by AD (Table 7). As illustrated in Figure 4A to 4E (magenta line), no significant associations were observed between plasma irisin and ROI volumes in non-AD status. In contrast, increased plasma irisin levels correlated significantly with lower ROI volumes when AD existed (Figure 4A to 4E, teal blue line).

Parameters in Figure 3 and Figure 4, including standardized beta values, were calculated based on the double moderation analyses (Table 7).

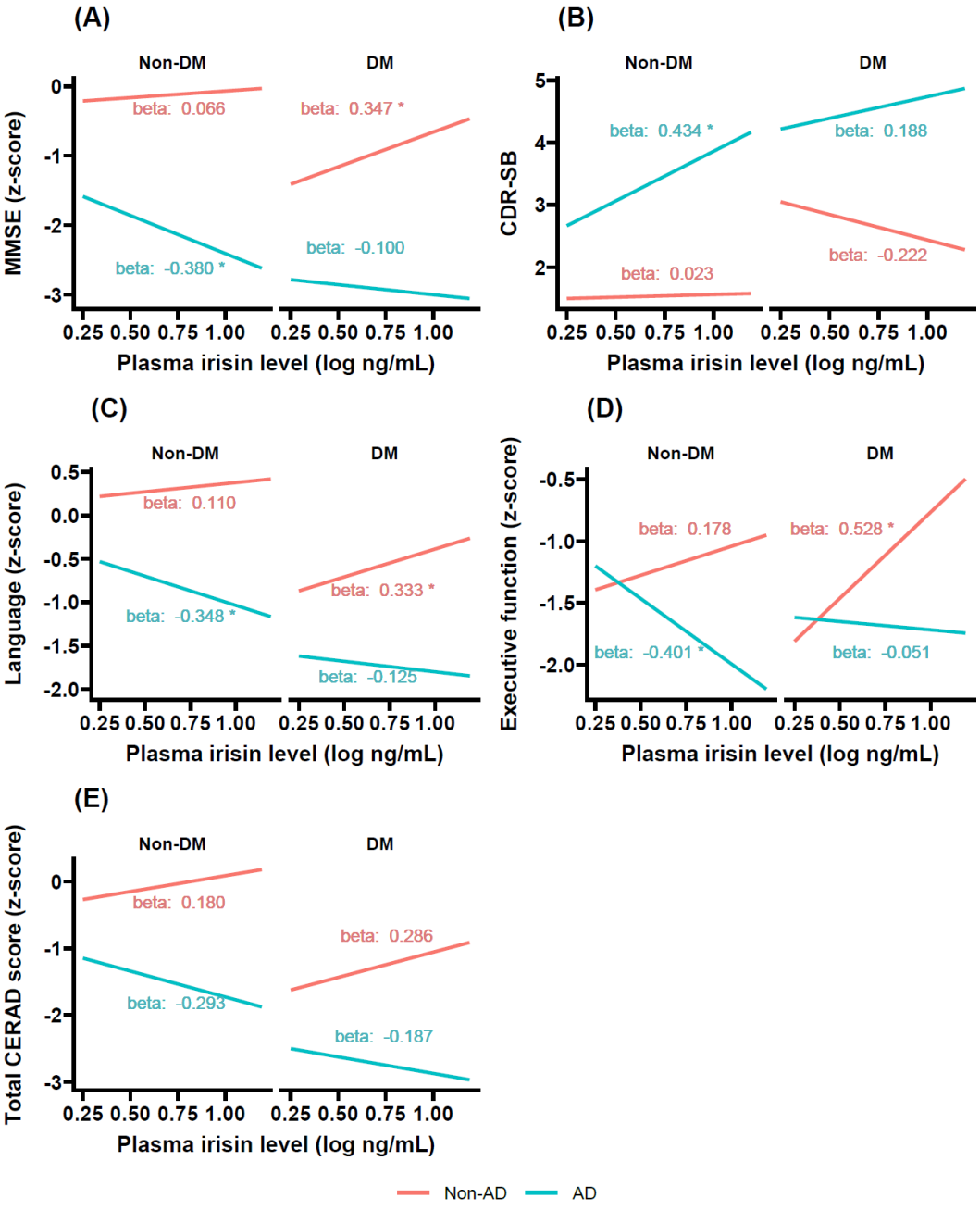

Figure 3. Conditional association between plasma irisin and cognition at different AD and DM status. [39] Presented values inside the plots are standardized betas for linear regression analyses.

Note that the association between irisin and cognitive function was altered by the presence of AD rather than the presence of DM.
* p value < 0.05
Abbreviations are explained on page viii.

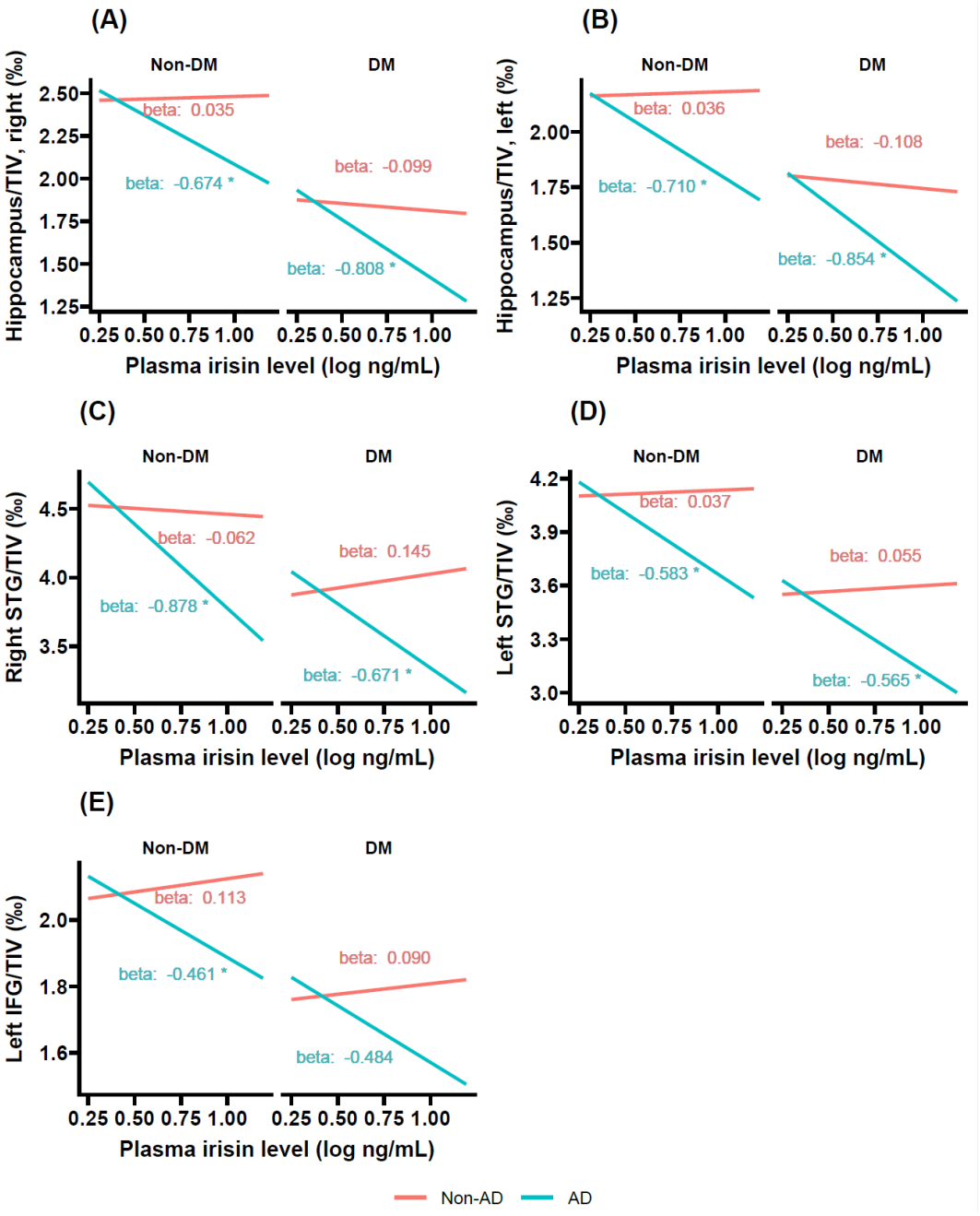

Figure 4. Conditional association between plasma irisin and ROI volume at different AD and DM status. [39] Presented values inside the plots are standardized betas for linear regression analyses.

Note that the association between irisin and cognitive function was altered by the presence of AD rather than the presence of DM.
* p value < 0.05
Abbreviations are explained on page viii.

3.9. Moderating effect of AD and DM on association between plasma irisin and cognition mediated by hippocampus: moderated mediation analysis

Mediation models were established to explore the role of hippocampal volume as a mediator between plasma irisin and cognition, while also examining the moderating effects of AD and DM on this relationship (Figure 5). Hippocampal volume, serving as the mediator, significantly influenced MMSE, CDR-SB, memory, language, executive function, and total CERAD-K. The mediating effect of hippocampal volume between plasma irisin and cognitive outcomes was moderated by AD status (Figures 5A, 5B, 5C, 5D, 5G, and 5H). However, the direct effect of plasma irisin on these cognitive outcomes was not significant (Figures 5A, 5B, 5C, 5D, 5G, and 5H). Plasma irisin levels did not directly or indirectly explain variations in visuospatial function and attention (Figures 5E and 5F).

The conditional indirect effects of plasma irisin levels via hippocampal volume, stratified by AD and DM statuses, are detailed in Table 8. For MMSE, memory, and total CERAD-K, the indirect effect was significant in the presence of AD, irrespective of DM status. Notably, the standardized beta values were negative, indicating that poorer performance in MMSE, memory, and total CERAD-K were indirectly associated with increased plasma irisin levels. Among participants with AD but without DM, significant indirect effects were observed for language and executive function.

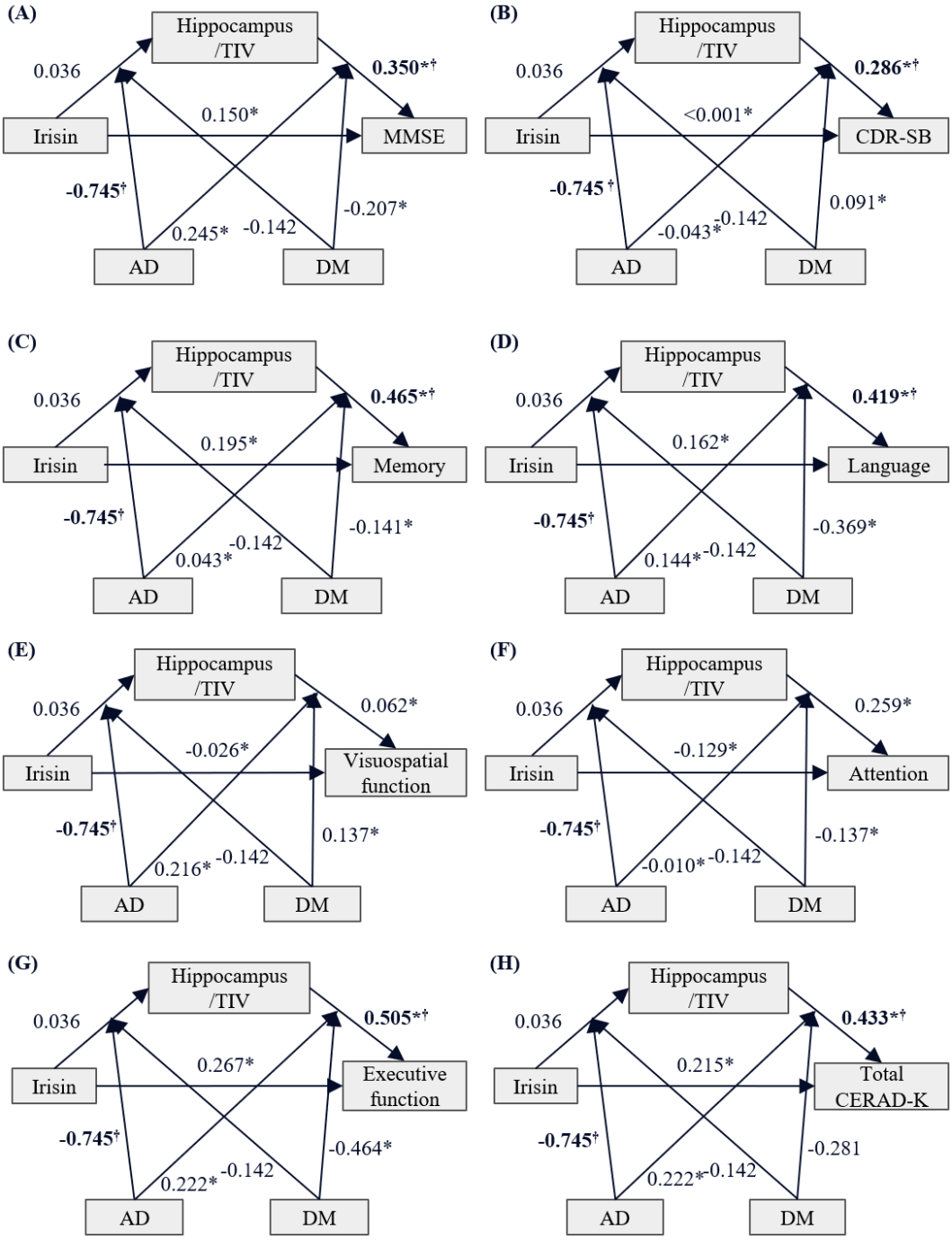

Figure 5. Results of moderated mediation models.

Presented numbers are standardized beta.
*P values were adjusted for FDR using Benjamini-Hochberg procedure.
†p value or adjusted p value <0.05 (also marked in bold sign)
Abbreviations are explained on page viii.

Table 8. Conditional indirect effect of irisin on cognition via hippocampus: results of moderated mediation analyses

Outcome	AD status	DM status	Standardized beta	BoootSE	BootLLCI	BootULCI
MMSE	Absent	Absent	0.013	0.044	-0.084	0.101
	Absent	Present	-0.015	0.042	-0.097	0.082
	Present	Absent	**-0.422**	**0.158**	**-0.772**	**-0.157**
	Present	Present	**-0.330**	**0.154**	**-0.652**	**-0.040**
CDR-SB	Absent	Absent	-0.010	0.037	-0.087	0.067
	Absent	Present	0.021	0.058	-0.090	0.147
	Present	Absent	0.233	0.174	-0.116	0.582
	Present	Present	0.202	0.180	-0.103	0.622
Memory	Absent	Absent	0.017	0.057	-0.098	0.139
	Absent	Present	-0.034	0.068	-0.187	0.089
	Present	Absent	**-0.360**	**0.173**	**-0.794**	**-0.117**
	Present	Present	**-0.312**	**0.161**	**-0.684**	**-0.043**
Language	Absent	Absent	0.015	0.052	-0.098	0.118
	Absent	Present	-0.005	0.044	-0.087	0.101
	Present	Absent	**-0.399**	**0.184**	**-0.792**	**-0.064**
	Present	Present	-0.165	0.185	-0.558	0.202
Visuospatial function	Absent	Absent	0.002	0.020	-0.034	0.053
	Absent	Present	-0.021	0.060	-0.162	0.084
	Present	Absent	-0.197	0.164	-0.552	0.083
	Present	Present	-0.353	0.229	-0.880	0.035
Attention	Absent	Absent	0.009	0.034	-0.061	0.082
	Absent	Present	-0.013	0.051	-0.132	0.076
	Present	Absent	-0.106	0.161	-0.448	0.216
	Present	Present	-0.011	0.234	-0.598	0.322
Executive function	Absent	Absent	0.018	0.062	-0.098	0.151
	Absent	Present	-0.004	0.052	-0.084	0.136
	Present	Absent	**-0.515**	**0.203**	**-0.990**	**-0.195**
	Present	Present	-0.224	0.137	-0.528	0.016
Total CERAD-K	Absent	Absent	0.016	0.052	-0.093	0.123
	Absent	Present	-0.016	0.050	-0.126	0.080
	Present	Absent	**-0.464**	**0.181**	**-0.884**	**-0.173**
	Present	Present	**-0.318**	**0.182**	**-0.710**	**0.000**

Direct effect of irisin on cognition is presented in Figure 5.
Hippocampus/TIV was the sum of the right and left hippocampus/TIV.
Bold signal means statistical significance
Level of confidence for all confidence intervals in output was 95%
Number of bootstraps for percentile bootstrap confidence intervals: 5000
Abbreviations are explained on page viii.

3.10. Predictive value of plasma irisin on cognition

The linear mixed-effect models revealed that baseline plasma irisin levels were not associated with changes in cognitive function outcomes in our study participants (Table 9). Furthermore, the presence or absence of AD and DM did not modify these longitudinal associations between baseline plasma irisin levels and cognitive outcomes (Table 10).

Table 9. Predictive value of plasma irisin on cognitive decline

Outcome	Unstandardized beta	SE	t value	p value	Adjusted p value
MMSE	-0.008	0.007	-1.030	0.312	0.625
CDR-SB	0.021	0.018	1.200	0.240	0.625
Memory	-0.006	0.004	-1.423	0.159	0.625
Language	-0.001	0.003	-0.463	0.648	0.744
Visuospatial function	-0.001	0.003	-0.412	0.686	0.744
Attention	0.004	0.007	0.574	0.576	0.744
Executive function	-0.002	0.005	-0.329	0.744	0.744
Total CERAD-K	-0.005	0.005	-1.123	0.274	0.625

Presented values are linear mixed-effect model parameters of interaction term irisin × time (months).
Models were adjusted for age, sex, education level (years), SGDS, hypertension, smoking, alcohol abuse, BMI, HbA1c, APOE4, and exercise frequency.
Adjusted p values were corrected for FDR using Benjamini-Hochberg procedure.
Abbreviations are explained on page viii.

Table 10. Impact of AD or DM on the predictive value of plasma irisin

Outcome	Unstandardized beta	SE	t value	p value	Adjusted p value
Impact of AD					
MMSE	-0.017	0.016	-1.061	0.297	0.731
CDR-SB	0.068	0.034	1.965	0.058	0.466
Memory	0.002	0.009	0.199	0.843	0.843
Language	-0.007	0.007	-0.923	0.365	0.731
Visuospatial function	-0.007	0.007	-1.147	0.269	0.731
Attention	0.008	0.019	0.404	0.693	0.792
Executive function	0.006	0.013	0.488	0.627	0.792
Total CERAD-K	-0.008	0.011	-0.703	0.489	0.782
Impact of DM					
MMSE	0.017	0.017	0.964	0.345	0.459
CDR-SB	-0.073	0.041	-1.786	0.086	0.437
Memory	0.007	0.009	0.770	0.443	0.507
Language	0.009	0.007	1.296	0.211	0.437
Visuospatial function	-0.008	0.006	-1.285	0.218	0.437
Attention	0.010	0.017	0.569	0.580	0.580
Executive function	-0.019	0.012	-1.562	0.125	0.437
Total CERAD-K	0.011	0.011	1.031	0.315	0.459

Presented values are linear mixed-effect model parameters of interaction term irisin × time (months) × AD or DM.
Models were adjusted for age, sex, education level (years), SGDS, hypertension, smoking, alcohol abuse, BMI, HbA1c, APOE4, and exercise frequency.
Adjusted p values were corrected for FDR using Benjamini-Hochberg procedure.
Abbreviations are explained on page viii.

4. DISCUSSION

This study sought to understand how plasma irisin levels relate to cognitive function and brain volume, regarding the roles of AD and DM as potential moderating factors (double moderation analysis). This study also examined the mediation effect of hippocampal volume on the irisin-cognition relationship under the influence of AD and DM (moderated mediation analysis). It further assessed the biomarker potential of plasma irisin for predicting cognitive decline.

The double moderation models showed that AD, rather than DM, significantly affected the cross-sectional association between plasma irisin and cognitive performance. Increased plasma irisin levels were associated with better cognitive function, particularly in the non-AD group (CN+MCI). Furthermore, in participants with AD, higher plasma irisin levels were associated with reduced ROI volumes. These results have been published as a part of this study by the same author. [39] This thesis additionally reports the results of moderated mediation models that hippocampal volume mediated the irisin-cognition relationship, with AD serving as a modifier of this indirect effect. In longitudinal analyses, plasma irisin did not predict changes in cognition, regardless of AD or DM status.

4.1. Plasma irisin levels across different cognitive and diabetic statuses

Participants with CN had higher plasma irisin levels compared to those with MCI or AD (Table 1), suggesting a potential neuroprotective role of irisin. Yet, previous studies reported mixed results. Aligning with the present study findings, prior research presented that individuals with vascular dementia had lower plasma irisin levels than healthy controls. [31] However, some studies found no significant difference in plasma irisin levels between individuals with CN and AD, [26,57] and one study even reported lower plasma irisin levels in the CN group compared to the MCI group. [33] Given these conflicting findings and the potential link between irisin deficiency and AD pathology, [26,27,58] the role of plasma irisin as a biomarker for neurodegeneration needs to be further investigated.

4.2. Impact of AD on the association of plasma irisin with cognition and ROI volume: proposing the concept of irisin resistance

Participants with MCI in this study exhibited poorer cognitive function but were less likely to carry APOE ε4 allele compared to those with CN, indicating homogeneity between MCI and CN groups. Therefore, it is plausible to consider participants with MCI and CN together as those without AD in the stratified multiple regression, double moderation, moderated mediation, and linear mixed-effect analyses.

4.2.1. Cognitive function and plasma irisin in the context of AD

Increased plasma irisin levels were associated with better cognitive function mainly in participants without AD, although statistical significance was lost after FDR correction. In contrast, increased plasma irisin levels were associated with poorer executive function in participants with AD (Table 3). The double moderation analysis revealed that AD notably influenced the relationship between plasma irisin and cognitive function; in participants without AD, plasma irisin level positively correlated with cognitive function; conversely, this correlation was absent or even inverted in those with AD (Table 7 and Figure 3). Given the irisin's known neuroprotective properties, [25,26] the absence of a correlation between plasma irisin and cognition in participants with AD seems to be counterintuitive or paradoxical. These observations suggest that circulating irisin may fail to exert its typical function under advanced neurodegenerative conditions such as AD. Evidence supporting this speculation comes from findings showing a lower ratio of irisin in CSF to plasma in patients with AD. [26] This implies that the efficacy of systemic circulating irisin on the brain may be compromised under pathologic neurodegenerative stress, or that blood level of irisin may increase in response to brain pathology.

4.2.2. Brain structure and plasma irisin in the context of AD

The relationship of plasma irisin levels with the volumes of ROIs varied with cognitive status. Plasma irisin levels were inversely correlated with the volumes of left hippocampus, bilateral STG, and left IFG in participants with AD. However, this correlation was not significant in those without AD (Table 4). The double moderation analysis demonstrated that AD significantly influenced the relationship between plasma irisin levels and the volumes of bilateral hippocampus, bilateral STG, and left IFG (Table 7 and Figure 4). These findings, together with the results of non-significant relationship between plasma irisin and cognition in AD, support the proposed concept of "irisin resistance", which suggests that irisin may play a compensatory role against neurodegeneration, as previously suggested. [24,26,27,33] This speculation is consistent with earlier results showing that, in individuals with CN, elevated CSF irisin levels were associated with aging, [26,59] whereas this association was not observed in those with AD. [26] Such findings imply that irisin may increase in response to age-related neurodegeneration, but this compensatory mechanism may be inadequate or dysfunctional in patients with AD.

4.2.3. Possible mechanism of irisin resistance

As mentioned above, loss of association between plasma irisin and cognition in AD, alongside the inverse association between plasma irisin and brain structure, led to the concept of irisin resistance. Irisin, circulating in the bloodstream, originates from the membrane protein FNDC5 in skeletal muscle, [19] under the influence of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) and estrogen-related receptor α (Errα). [25] When irisin reaches the brain through the blood-brain barrier, [60] it triggers cyclic adenosine monophosphate (cAMP) – protein kinase A (PKA) – cAMP-responsive element-binding protein (CREB) pathway in the hippocampus. [25,26] In the pathologic condition of AD, this irisin-mediated neuroprotective signaling may be compromised at a specific point. Alternatively, irisin may be produced in the hippocampus itself, and AD pathology could impair this local irisin, potentially leading to an increase in peripheral circulating irisin to compensate. This speculation is supported by a recent study showing that irisin treatment markedly reduced Aβ pathology. [27]

4.2.4. Influence of irisin on cognitive function: hippocampal mediation

The moderated mediation analysis in this study revealed that hippocampal volume partially mediated the relationship between plasma irisin levels and cognitive function in participants with AD (Figure 5). Under mediation by hippocampal volume, increased plasma irisin levels were associated with poorer cognitive performance in the presence of AD (Table 8). This supports the hypothesis of irisin resistance, suggesting that a decrease in hippocampal volume, alongside impaired cognitive function, may trigger an increase in circulating irisin levels in response to advanced neurodegenerative conditions such as AD. Sufficient evidence points to the hippocampus as a primary target for irisin's effect. Irisin is found predominantly in the hippocampus of both humans and mice, [25,26] and its levels are decreased in the brain of AD mouse models as well as in human AD patients. [26,60] Furthermore, physical exercise is known to upregulate the expression of *Fndc5* gene and FNDC5 protein in the hippocampus of mice. [25,61] The significance of the present study lies in validating the role of the hippocampus as a central site of irisin's action, as demonstrated through human data. Future studies should explore whether the administration of irisin to humans could potentially ameliorate hippocampal atrophy or neurodegeneration.

4.3. Impact of DM on association of plasma irisin levels with cognition and ROI volume

This study investigated whether DM also affects the relationship of plasma irisin with cognitive function and brain structure, in addition to the recognized impact of AD. Irisin's anti-diabetic and neuroprotective properties have been well established. [19] Given the detrimental effect of DM on cognitive decline, [6] it was expected that diabetic conditions would affect the relationship between plasma irisin and cognitive function; a positive association between irisin and cognition could be either enhanced in diabetic condition via indirect anti-diabetic pathway or attenuated as irisin's effect on cognition might be compromised in diabetic conditions.

However, the results of this study showed that DM, contrary to AD, did not

significantly alter the association between plasma irisin levels and cognitive outcomes (Table 7 and Figure 3 to 5). Patients with DM experience insulin resistance even in the brain as well as in the systemic circulation. [62,63] However, unlike insulin, irisin seems to have a positive effect still under diabetic conditions; previous studies have indicated that irisin administration in diabetic rats or mice can enhance neurotrophic effects on the brain[37] and ameliorate atherosclerosis. [64] Therefore, the results of this study imply that, unlike insulin, the brain's responsiveness to irisin may be preserved in the presence of DM.

Another possible explanation is that the significant moderating effect of AD may have masked the influence of DM. A substantial portion of participants with AD in DM (12 of 32 [37.5%]) and non-DM (23 of 75 [30.7%]) groups might affect the results of DM. This speculation is supported by the conditional subgroup analyses of double moderation models; among participants without AD, increased plasma irisin levels were associated with better performances in MMSE, language, and executive function in those with DM, while these relationships were not observed in those without DM (Figure 3A, 3C, and 3D). Additional comprehensive studies with larger sample sizes are required to ascertain the exact interplay among DM, irisin, and the brain.

4.4. Longitudinal association between baseline plasma irisin and cognitive decline over time

Linear mixed-effect models in this study did not demonstrate a significant link between baseline plasma irisin levels and subsequent changes in cognitive function (Table 9), irrespective of the presence of AD or DM (Table 10). This finding suggests that plasma irisin may not serve as a reliable indicator for predicting cognitive decline. To date, no longitudinal studies have been published to specifically address the predictive value of irisin for cognitive decline. Previous studies with follow-up periods ranging from 3 to 6 months have noted that lower serum irisin levels corresponded with worse functional outcomes, such as motor and sensory abilities, and increased mortality in acute stroke patients. [65,66] These studies, however, explored relatively short-term effects. In contrast, the mean follow-up period of the present study was 42.3 months, potentially explaining the observed discrepancies. It is worth noting that irisin has neurotrophic properties, unlike other biomarkers of neurodegeneration, such as neurofilament light chain or Aβ, which are byproducts of pathological processes.

4.5. Limitations

This study requires careful consideration when interpreting the results. First, significant differences in baseline education levels between the three cognitive groups could have introduced bias into the findings. Although adjustments were made for education level and cognitive test scores were standardized according to age, sex, and education, future studies with a larger cohort can stratify participants based on their education levels to further clarify this issue. Second, the sample size was relatively small in cross-sectional analyses given the number of explanatory variables, although the covariates were clinically relevant to outcomes (cognitive function or brain structure). Third, the cross-sectional and observational design of the moderation and moderated mediation analyses limits the ability to determine the causal direction. Although this study investigated the longitudinal changes in cognitive function, follow-up data on brain MRI scans and plasma irisin levels are required to fully understand their interaction. To confirm the proposed hypothesis of irisin resistance, tracking plasma irisin levels in individuals through normal and pathologic aging is essential. Fourth, a more detailed investigation into the relationship between irisin and AD pathology or brain structure may benefit from measuring levels of irisin, BDNF, and Aβ both in plasma and CSF. Fifth, the exercise data in this study was based solely on self-reported frequency. Future studies are needed to explore the relationship between plasma irisin, muscle mass measured by dual-energy X-ray absorptiometry or bioelectrical impedance analysis, and cognitive changes. Last, cognitive frailty, the concept including sarcopenia and MCI, has been consistently suggested as a potentially reversible prodromal status of dementia. [67] Therefore, it would be beneficial for future studies to investigate whether irisin plays a favoring role in the relationship between cognitive frailty and dementia.

5. CONCLUSION

This study highlighted the discrepancies in the association of plasma irisin with cognition and brain structure regarding the clinical diagnosis of AD; in the case of AD, the positive association between plasma irisin levels and cognitive function disappeared, and increased plasma irisin levels were associated with reduced brain volume. When considering the hippocampus as a mediator, an inverse association was observed between plasma irisin and cognition, but only in the presence of AD. These findings imply that the positive impact of plasma irisin on cognition may vanish in AD, supporting the hypothesis of a compensatory role for irisin in response to neurodegeneration and introducing the concept of "irisin resistance." Physical exercise may be effective in delaying cognitive decline, particularly during the preclinical or prodromal AD stage. Given the emerging therapeutic capacity of irisin, its pro-cognitive effects may be more pronounced in the earlier stage of the disease. On the other hand, DM did not have a significant impact on the association of plasma irisin with cognitive function and brain structure. Longitudinal analyses indicate that plasma irisin does not have predictive potential for cognitive changes, irrespective of AD and DM statuses.

References

1. Vital Statistics Division Statistics Korea, Noh H, Seo J, Lee S, Yi N, Park S, et al. Cause-of-death statistics in 2020 in the Republic of Korea. J Korean Med Assoc 2023;66:132-42.
2. World Health Organization. The top 10 causes of death [Accessed 8 January 2024]
3. Wimo A, Seeher K, Cataldi R, Cyhlarova E, Dielemann JL, Frisell O, et al. The worldwide costs of dementia in 2019. Alzheimer's & Dementia 2023;19:2865-73.
4. Jack CR, Jr., Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. The Lancet Neurology 2010;9:119-28.
5. Jack CR, Jr. Advances in Alzheimer's disease research over the past two decades. The Lancet Neurology 2022;21:866-9.
6. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet 2020;396:413-46.
7. Knopman DS, Hershey L. Implications of the Approval of Lecanemab for Alzheimer Disease Patient Care. Neurology 2023;101:610-20.
8. Baker LD, Frank LL, Foster-Schubert K, Green PS, Wilkinson CW, McTiernan A, et al. Effects of aerobic exercise on mild cognitive impairment: a controlled trial. Arch Neurol 2010;67:71-9.
9. Okonkwo OC, Schultz SA, Oh JM, Larson J, Edwards D, Cook D, et al. Physical activity attenuates age-related biomarker alterations in preclinical AD. Neurology 2014;83:1753-60.
10. Blondell SJ, Hammersley-Mather R, Veerman JL. Does physical activity prevent cognitive decline and dementia?: A systematic review and meta-analysis of longitudinal studies. BMC Public Health 2014;14:510.
11. Raichlen DA, Aslan DH, Sayre MK, Bharadwaj PK, Ally M, Maltagliati S, et al. Sedentary Behavior and Incident Dementia Among Older Adults. JAMA 2023;330:934-40.
12. World Health Organization. Risk reduction of cognitive decline and dementia: WHO guidelines. 2019.
13. Colcombe SJ, Erickson KI, Scalf PE, Kim JS, Prakash R, McAuley E, et al. Aerobic exercise training increases brain volume in aging humans. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 2006;61:1166-70.
14. Sobol NA, Hoffmann K, Frederiksen KS, Vogel A, Vestergaard K, Brændgaard H, et al. Effect of aerobic exercise on physical performance in patients with Alzheimer's disease. Alzheimers Dement 2016;12:1207-15.
15. Colberg SR, Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, et al. Exercise and Type 2 Diabetes: The American College of Sports Medicine and the American Diabetes Association: joint position statement. Diabetes Care 2010;33:e147-e67.

16. Isaac AR, Lima-Filho RAS, Lourenco MV. How does the skeletal muscle communicate with the brain in health and disease? Neuropharmacology 2021;197:108744.
17. Cotman C. Exercise: a behavioral intervention to enhance brain health and plasticity. Trends Neurosci 2002;25:295-301.
18. Vaynman S, Ying Z, Gomez-Pinilla F. Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. Eur J Neurosci 2004;20:2580-90.
19. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 2012;481:463-8.
20. Löffler D, Müller U, Scheuermann K, Friebe D, Gesing J, Bielitz J, et al. Serum Irisin Levels Are Regulated by Acute Strenuous Exercise. The Journal of Clinical Endocrinology & Metabolism 2015;100:1289-99.
21. Jedrychowski Mark P, Wrann Christiane D, Paulo Joao A, Gerber Kaitlyn K, Szpyt J, Robinson Matthew M, et al. Detection and Quantitation of Circulating Human Irisin by Tandem Mass Spectrometry. Cell Metab 2015;22:734-40.
22. Lee P, Linderman Joyce D, Smith S, Brychta Robert J, Wang J, Idelson C, et al. Irisin and FGF21 Are Cold-Induced Endocrine Activators of Brown Fat Function in Humans. Cell Metab 2014;19:302-9.
23. Perakakis N, Triantafyllou GA, Fernandez-Real JM, Huh JY, Park KH, Seufert J, Mantzoros CS. Physiology and role of irisin in glucose homeostasis. Nat Rev Endocrinol 2017;13:324-37.
24. Young MF, Valaris S, Wrann CD. A role for FNDC5/Irisin in the beneficial effects of exercise on the brain and in neurodegenerative diseases. Prog Cardiovasc Dis 2019;62:172-8.
25. Wrann CD, White JP, Salogiannnis J, Laznik-Bogoslavski D, Wu J, Ma D, et al. Exercise induces hippocampal BDNF through a PGC-1α/FNDC5 pathway. Cell Metab 2013;18:649-59.
26. Lourenco MV, Frozza RL, de Freitas GB, Zhang H, Kincheski GC, Ribeiro FC, et al. Exercise-linked FNDC5/irisin rescues synaptic plasticity and memory defects in Alzheimer's models. Nat Med 2019;25:165-75.
27. Kim E, Kim H, Jedrychowski MP, Bakiasi G, Park J, Kruskop J, et al. Irisin reduces amyloid-beta by inducing the release of neprilysin from astrocytes following downregulation of ERK-STAT3 signaling. Neuron 2023;111:3619-33 e8.
28. Lourenco MV, Ribeiro FC, Sudo FK, Drummond C, Assunção N, Vanderborght B, et al. Cerebrospinal fluid irisin correlates with amyloid-β, BDNF, and cognition in Alzheimer's disease. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring 2020;12:e12034.
29. Küster OC, Laptinskaya D, Fissler P, Schnack C, Zügel M, Nold V, et al. Novel Blood-Based Biomarkers of Cognition, Stress, and Physical or Cognitive Training in Older Adults at Risk of Dementia: Preliminary Evidence for a Role of BDNF, Irisin, and the Kynurenine Pathway. J Alzheimers Dis 2017;59:1097-111.

30. Tsai C-L, Pai M-C. Circulating levels of Irisin in obese individuals at genetic risk for Alzheimer's disease: Correlations with amyloid-β, metabolic, and neurocognitive indices. Behav Brain Res 2021;400:113013.
31. Zhang F, Hou G, Hou G, Wang C, Shi B, Zheng Y. Serum Irisin as a Potential Biomarker for Cognitive Decline in Vascular Dementia. Front Neurol 2021;12.
32. Conti E, Grana D, Stefanoni G, Corsini A, Botta M, Magni P, et al. Irisin and BDNF serum levels and behavioral disturbances in Alzheimer's disease. Neurol Sci 2019;40:1145-50.
33. Lin H, Yuan Y, Tian S, Han J, Huang R, Guo D, et al. In Addition to Poor Glycemic Control, a High Level of Irisin in the Plasma Portends Early Cognitive Deficits Clinically in Chinese Patients With Type 2 Diabetes Mellitus. Front Endocrinol (Lausanne) 2019;10:634.
34. Hee Park K, Zaichenko L, Brinkoetter M, Thakkar B, Sahin-Efe A, Joung KE, et al. Circulating Irisin in Relation to Insulin Resistance and the Metabolic Syndrome. The Journal of Clinical Endocrinology & Metabolism 2013;98:4899-907.
35. Arvanitakis Z, Wilson RS, Bienias JL, Evans DA, Bennett DA. Diabetes mellitus and risk of Alzheimer disease and decline in cognitive function. Arch Neurol 2004;61:661-6.
36. Lourenco MV, de Freitas GB, Raony Í, Ferreira ST, De Felice FG. Irisin stimulates protective signaling pathways in rat hippocampal neurons. Front Cell Neurosci 2022;16:953991.
37. Huang L, Yan S, Luo L, Yang L. Irisin regulates the expression of BDNF and glycometabolism in diabetic rats. Mol Med Rep 2019;19:1074-82.
38. Blennow K, Galasko D, Perneczky R, Quevenco F-C, van der Flier WM, Akinwonmi A, et al. The potential clinical value of plasma biomarkers in Alzheimer's disease. Alzheimers Dement 2023;n/a.
39. Kim KY, Kwak S, Ha J, Oh DJ, Kim M, Cho SY, et al. Loss of association between plasma irisin levels and cognition in Alzheimer's disease. Psychoneuroendocrinology 2022;136:105624.
40. Lourenco MV, Frozza RL, de Freitas GB, Zhang H, Kincheski GC, Ribeiro FC, et al. Exercise-linked FNDC5/irisin rescues synaptic plasticity and memory defects in Alzheimer's models. Nat Med 2019;25:165-75.
41. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189-98.
42. Lee JH, Lee KU, Lee DY, Kim KW, Jhoo JH, Kim JH, et al. Development of the Korean Version of the Consortium to Establish a Registry for Alzheimer's Disease Assessment Packet (CERAD-K) clinical and neuropsychological assessment batteries. 2002;57:P47-P53.
43. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack Jr CR, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:263-9.
44. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Kokmen E, Tangelos EG. Aging, memory, and mild cognitive impairment. Int Psychogeriatr 1997;9:65-9.

45. care ADAJD. Diagnosis and classification of diabetes mellitus. 2010;33:S62-S9.
46. Hixson JE, Vernier DTJJolr. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. 1990;31:545-8.
47. Lee DY, Lee KU, Lee JH, Kim KW, Jhoo JH, Kim SY, et al. A normative study of the CERAD neuropsychological assessment battery in the Korean elderly. Journal of the international neuropsychological society: JINS 2004;10:72.
48. Seo EH, Lee DY, Kim KW, Lee JH, Jhoo JH, Youn JC, et al. A normative study of the Trail Making Test in Korean elders. International Journal of Geriatric Psychiatry: A journal of the psychiatry of late life and allied sciences 2006;21:844-52.
49. van Dyck CH, Swanson CJ, Aisen P, Bateman RJ, Chen C, Gee M, et al. Lecanemab in Early Alzheimer's Disease. N Engl J Med 2023;388:9-21.
50. Jeon SY, Byun MS, Yi D, Lee JH, Choe YM, Ko K, et al. Influence of hypertension on brain amyloid deposition and Alzheimer's disease signature neurodegeneration. Neurobiol Aging 2019;75:62-70.
51. Tsai J-Z, Peng S-J, Chen Y-W, Wang K-W, Li C-H, Wang J-Y, et al. Automated segmentation and quantification of white matter hyperintensities in acute ischemic stroke patients with cerebral infarction. PLoS One 2014;9:e104011.
52. Kochunov P, Lancaster JL, Thompson P, Woods R, Mazziotta J, Hardies J, Fox P. Regional Spatial Normalization: Toward an Optimal Target. J Comput Assist Tomogr 2001;25:805-16.
53. Fox NC, Warrington EK, Freeborough PA, Hartikainen P, Kennedy AM, Stevens JM, Rossor MN. Presymptomatic hippocampal atrophy in Alzheimer's disease: A longitudinal MRI study. Brain 1996;119:2001-7.
54. Provenzano FA, Muraskin J, Tosto G, Narkhede A, Wasserman BT, Griffith EY, et al. White matter hyperintensities and cerebral amyloidosis: necessary and sufficient for clinical expression of Alzheimer disease? JAMA Neurol 2013;70:455-61.
55. Bae JN, Cho MJ. Development of the Korean version of the Geriatric Depression Scale and its short form among elderly psychiatric patients. J Psychosom Res 2004;57:297-305.
56. Hayes AF. Introduction to Mediation, Moderation, and Conditional Process Analysis Third Edition: A Regression-Based Approach; 2022.
57. Conti E, Grana D, Stefanoni G, Corsini A, Botta M, Magni P, et al. Irisin and BDNF serum levels and behavioral disturbances in Alzheimer’s disease. Neurol Sci 2019;40:1145-50.
58. de Freitas GB, Lourenco MV, De Felice FG. Protective actions of exercise-related FNDC5/Irisin in memory and Alzheimer’s disease. J Neurochem 2020;155:602-11.
59. Ruan Q, Huang Y, Yang L, Ruan J, Gu W, Zhang X, et al. The effects of both age and sex on irisin levels in paired plasma and cerebrospinal fluid in healthy humans. Peptides 2019;113:41-51.
60. Islam MR, Valaris S, Young MF, Haley EB, Luo R, Bond SF, et al. Exercise hormone irisin is a critical regulator of cognitive function. Nat Metab 2021;3:1058-70.
61. Choi SH, Bylykbashi E, Chatila ZK, Lee SW, Pulli B, Clemenson GD, et al. Combined adult neurogenesis and BDNF mimic exercise effects on cognition in an Alzheimer’s mouse

model. Science (1979) 2018;361:eaan8821.
62. An Y, Varma VR, Varma S, Casanova R, Dammer E, Pletnikova O, et al. Evidence for brain glucose dysregulation in Alzheimer's disease. Alzheimers Dement 2018;14:318-29.
63. Arnold SE, Arvanitakis Z, Macauley-Rambach SL, Koenig AM, Wang H-Y, Ahima RS, et al. Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums. Nat Rev Neurol 2018;14:168-81.
64. Zhu D, Wang H, Zhang J, Zhang X, Xin C, Zhang F, et al. Irisin improves endothelial function in type 2 diabetes through reducing oxidative/nitrative stresses. J Mol Cell Cardiol 2015;87:138-47.
65. Tu W-J, Qiu H-C, Cao J-L, Liu Q, Zeng X-W, Zhao J-Z. Decreased Concentration of Irisin Is Associated with Poor Functional Outcome in Ischemic Stroke. Neurotherapeutics 2018;15:1158-67.
66. Wu H, Guo P, Jin Z, Li X, Yang X, Tang C, et al. Serum levels of irisin predict short-term outcomes in ischemic stroke. Cytokine 2019;122:154303.
67. Sugimoto T, Arai H, Sakurai T. An update on cognitive frailty: Its definition, impact, associated factors and underlying mechanisms, and interventions. Geriatr Gerontol Int 2022;22:99-109.

ABSTRACT IN KOREAN

다양한 알츠하이머 치매 및 당뇨 상태에 따른 아이리신과 인지기능의 연관성

연구배경 및 목적: 신체 운동은 알츠하이머 병과 당뇨의 진행을 늦추는 것으로 알려져 있다. 뇌와 근육의 신호를 전달하는 핵심물질로 아이리신(irisin)이 주목받고 있는데, 아이리신은 인지기능 및 대사기능을 향상시키는 것으로 알려져 있다. 그러나 알츠하이머 치매와 당뇨의 상태에 따라 혈중 아이리신 농도와 인지기능 사이의 관계는 아직 명확하지 않다. 이 연구는 해마의 매개 역할에 초점을 맞춰 다양한 알츠하이머 치매와 당뇨의 상태에서 아이리신과 인지기능의 관계를 횡적으로 조사하였다. 또한, 종적으로 기저 혈중 아이리신 농도가 인지기능 저하를 예측하는지도 조사하였다.

연구방법: 본 연구에서는 50 세 이상의 참가자 107 명을 인지상태(인지정상 23 명, 경도인지장애 49 명, 알츠하이머 치매 35 명) 또는 당뇨 상태(비당뇨 75 명, 당뇨 32 명)에 따라 분류하였다. 혈액은 하룻밤 금식 후 채혈하여 혈장 아이리신 농도를 분석하였다. 알츠하이머 치매 또는 신체 운동과 관련된 뇌 영역인 해마(hippocampus), 백질고강도(white matter hyperintensity), 앞띠이랑(anterior cingulate gyrus), 위관자이랑(superior temporal gyrus), 아래이마이랑(inferior frontal gyrus) 분석을 위해 뇌 자기공명영상을 촬영하였다. 인지기능은 평균 42.3 개월의 추적 관찰 기간동안 매년 평가되었다. 혈장 아이리신 농도와 인지기능 사이의 관계에 알츠하이머 치매와 당뇨가 미치는 영향을 알아보기 위해 이중 조절 분석(double moderation analysis)을, 이 관계에서 해마 용적의 매개효과를 알아보기 위해 조절된 매개 분석(moderated mediation analysis)을 사용하였다. 혈장 아이리신이 인지기능 변화를 예측하는지 알아보기 위해 선형혼합효과모델을 적용하였다.

결과: 이중 조절 분석 결과, 당뇨상태가 아닌 알츠하이머 치매 상태가 혈장 아이리신 농도와 인지기능 또는 뇌 구조 사이의 관계를 유의하게 변화시켰다. 구체적으로 살펴보면, 혈장 아이리신 농도가 높을수록 높은 인지기능을 보이는 관계는 알츠하이머 치매가 없는 참가자들, 즉 인지정상 또는 경도인지장애 군에서만 나타났고, 알츠하이머 치매를 가진 참가자에서는 이 관계가 나타나지 않았다. 또한, 알츠하이머 치매 군에서만 특이적으로 혈장 아이리신 농도가 높을수록 뇌 용적이 감소하였다. 조절된 매개 분석 모델에서는 해마 용적의 매개 하에 혈장 아이리신 농도의 증가가 인지기능 저하와 연관되었는데, 알츠하이머 치매 군에서만 이런 관련성이 나타났다. 한편, 기저의 혈장 아이리신 농도는

인지기능의 변화를 예측하지 못했으며, 이는 기저의 알츠하이머 치매와 당뇨의 상태에 관계가 없었다.

결론: 본 연구 결과는 혈장 아이리신이 인지기능에 미치는 긍정적인 영향이 알츠하이머 치매 상태에서는 사라질 수 있음을 시사한다. 또한, 아이리신이 점진적 신경퇴행에 반응하여 보상적인 역할을 할 수 있다는 아이리신 저항성 개념을 뒷받침한다.

핵심되는 말 : 아이리신, 인지기능, 알츠하이머 치매, 당뇨, 해마

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