

CEREBROSPINAL FLUID BIOMARKERS

Cerebrospinal fluid irisin correlates with amyloid- β , BDNF, and cognition in Alzheimer's disease

Mychael V. Lourenco¹ | Felipe C. Ribeiro¹ | Felipe K. Sudo² | Cláudia Drummond^{2,3} |
 Naima Assunção^{2,4} | Bart Vanderborght² | Fernanda Tovar-Moll^{2,4} | Paulo Mattos^{2,4,5} |
 Fernanda G. De Felice^{1,6,7} | Sergio T. Ferreira^{1,8}

¹Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro, Cidade Universitaria – Rio de Janeiro, Rio de Janeiro, Brazil

²D'Or Institute for Research and Education (IDOR), Rio de Janeiro, Rio de Janeiro, Brazil

³Department of Speech and Hearing Pathology, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

⁴Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

⁵Institute of Psychiatry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

⁶Department of Psychiatry, Queen's University, Kingston, Ontario, Canada

⁷Centre for Neurosciences Studies, Queen's University, Kingston, Ontario, Canada

⁸Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil

Correspondence

Sergio T. Ferreira, Institute of Biophysics Carlos Chagas Filho and Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro, Av. Carlos Chagas Filho, 373 Bldg C – Room C-31 – Cidade Universitaria, Rio de Janeiro, RJ 21941-902, Brazil. E-mail: ferreira@bioqmed.ufrj.br

Fernanda G. De Felice, Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro, Av. Carlos Chagas Filho, 373 Bldg H – Room H2-019 – Cidade Universitaria, Rio de Janeiro, RJ 21941-902, Brazil. E-mail: felice@bioqmed.ufrj.br

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Abstract

Introduction: Irisin is a novel hormone originally identified for its role as a regulator of peripheral metabolism and recently found to protect synapses and rescue memory in mouse models of Alzheimer's disease (AD). However, whether and how cerebrospinal fluid (CSF) irisin varies in relation to canonical AD biomarkers and cognition in humans remains unknown.

Methods: We determined CSF levels of irisin and brain-derived neurotrophic factor (BDNF) and examined their correlations with CSF amyloid beta ($A\beta$)₄₂, total tau, and Mini-Mental State Exam (MMSE) scores in a cohort comprising AD patients ($n = 14$) and non-demented controls (NDC; $n = 25$).

Results: CSF irisin correlated positively with BDNF, $A\beta$ ₄₂, and MMSE scores, but not with CSF total tau.

Discussion: Results indicate that CSF irisin and BDNF are directly correlated with $A\beta$ pathology and cognition in AD.

KEYWORDS

Alzheimer's disease, BDNF, cerebrospinal fluid, dementia, FNDC5, irisin

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1 | INTRODUCTION

Mounting evidence indicates that peripheral and brain metabolic dysfunction are intimately connected and play relevant roles in the pathophysiology of brain disorders, including Alzheimer's disease (AD).¹⁻⁶ Impaired brain action of hormones has been associated with AD.^{2,7}

Irisin is an exercise-induced hormone first identified as a regulator of adipose tissue metabolism.⁸ Subsequent work revealed that irisin is expressed in the brain and induces expression of brain-derived neurotrophic factor (BDNF) in the mouse hippocampus.^{9,10} We recently demonstrated that boosting brain levels of irisin protected synapses and memory in mouse models of AD.⁹ Importantly, irisin appears to mediate the brain benefits of physical exercise in AD models.⁹ However, whether central irisin correlates with AD biomarkers and cognition in humans is unknown. To address this question, we investigated CSF levels of irisin and its correlations with AD-linked biomarkers, BDNF, and cognition in AD and control individuals.

2 | METHODS

2.1 | Study approval and ethics

Experimental procedures involving human cerebrospinal fluid (CSF) were approved by the Committee for Research Ethics of Copa D'Or Hospital (protocol no. 47163715.0.0000.5249). Donors gave written informed consent for use of CSF. Samples were anonymized prior to analyses and measurements were performed in a blinded fashion by trained investigators. All studies have been performed according to international ethical regulations and standards.

2.2 | Study population

From a total of 225 subjects initially recruited at a memory clinic at D'Or Institute of Research and Education (IDOR) in Rio de Janeiro, Brazil, 39 met inclusion criteria for the current study (age ≥ 60 years; absence of other neurological disorders, or neurodevelopmental or genetic diseases; native Brazilian Portuguese speakers; formal education ≥ 8 years; no restriction for magnetic resonance imaging [MRI] studies). The cohort studied included both non-demented controls (NDCs) and AD patients; both groups were evaluated with the same extensive clinical, neuropsychological, and neuroimage investigation as described previously.¹¹ For demographics and biomarker details, see Table 1.

2.3 | CSF samples

CSF was collected by lumbar puncture performed around 11 a.m. in all cases to minimize circadian fluctuations in analyte levels. CSF was centrifuged, aliquoted, immediately frozen at -80°C , and stored in the IDOR biobank. Prior to assays, samples were thawed and kept on ice until use. Samples and calibrators were run in duplicate.

RESEARCH IN CONTEXT

1. Systematic review: Irisin is an exercise-induced hormone implicated as a modulator of brain function and whose levels are reduced in Alzheimer's disease. However, potential correlations between central irisin levels, cognition, and Alzheimer's disease (AD) pathology are still unknown. The authors reviewed the related literature using PubMed.
2. Interpretation: Results indicate positive correlations between cerebrospinal fluid (CSF) irisin and brain-derived neurotrophic factor (BDNF), amyloid beta ($A\beta$)₄₂, and cognitive performance.
3. Future directions: Future studies should replicate the current findings in additional cohorts and investigate potential associations with markers of synapse damage, CSF p-tau, and neuroimaging data, as well as longitudinal assessments along disease progression. Determination of specificity of CSF irisin changes for AD versus other neurological disorders appears warranted.

2.4 | Enzyme-linked immunosorbent assays

Amyloid beta ($A\beta$)₄₀, $A\beta$ ₄₂, and t-tau concentrations were measured using Euroimmun (Lübeck, Germany) enzyme-linked immunosorbent assay (ELISA) kits. Kits for irisin detection were from Phoenix Pharmaceuticals (EK-067-29). BDNF kits were from Abcam (ab99978). ApoE4 kits were from MBL Biosciences (7635).

2.5 | Statistics

Statistical analysis was performed using GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA). Data were initially checked for normal distribution using the D'Agostino & Pearson Omnibus normality test. Statistical significances of differences between patient groups were assessed using two-tailed Student's t-test, except as indicated. Only cases with corresponding complete data were included for correlation analyses. Correlations were established by Pearson's linear regression.

3 | RESULTS

3.1 | Population characteristics

Demographics and results from biomarker measurements are presented in Table 1. No differences in sex or apolipoprotein E (ApoE4) status were observed between AD and NDC groups, while age was higher in the AD group. AD patients presented clear cognitive impairment, as assessed by Mini-Mental State Exam (MMSE) scores. As expected, CSF

TABLE 1 Demographic, clinical, and biomarker characteristics of CSF donor subjects

	Non-demented controls (NDC)	Alzheimer's disease (AD)	t; χ^2 (P-value)
Sex, male/female	10/15	4/10	0.5094 (0.9168)
Age (y)	67.8 ± 4.8 (61-79)	74.2 ± 7.1 (60-85)	3.363 (0.0018)
MMSE	27.6 ± 1.2 (25-29)	20.9 ± 4.1 (14-26)	7.614 (<0.0001)
ApoE4, positive/negative	7/18	6/8	0.8914 (0.8275)
CSF A β_{40} (pg/mL)	3872 ± 1910 (760-8296)	4481 ± 1755 (2107-8113)	0.9834 (0.3318)
CSF A β_{42} , (pg/mL)	474.3 ± 182.1 (156-849.9)	259.6 ± 68.7 (168.8-403.5)	4.221 (0.0002)
CSF t-tau (pg/mL)	324 ± 118 (161.9-554)	583.1 ± 226.9 (229.6-1181)	4.580 (<0.0001)
CSF BDNF (pg/mL)	216.5 ± 130.3 (70.9-640.3)	128.6 ± 63.6 (17.6-228.5)	2.361 (0.0236)

Values are presented as means ± SD (range). Statistical significances are presented as t (P-value) from two-tailed unpaired Student's t test, except for sex and ApoE4, which were analyzed using the Chi-Square Test, χ^2 (P-value).

Abbreviations: A β_{40} , amyloid- β 1-40; A β_{42} , amyloid- β 1-42; AD, Alzheimer's disease; CSF, cerebrospinal fluid; MMSE, Mini-Mental State Exam; NDC, non-demented controls; SD, standard deviation; t-tau, total tau.

levels of A β_{42} , but not A β_{40} , were reduced and total tau (t-tau) was consistently increased in the AD group.

3.2 | Correlations among CSF irisin, cognitive performance, and AD biomarkers

We initially asked whether CSF irisin, which we previously found to be reduced in AD,⁹ correlated with cognitive performance and CSF biomarkers in AD patients. Interestingly, CSF irisin showed significant positive correlations with MMSE scores (Figure 1A) and with CSF A β_{42} (Figure 1B). No correlation was found between irisin and total tau (a marker of neurodegeneration) in the CSF (Figure 1C). These results indicate that the reduction in CSF irisin parallels brain A β deposition, rather than reflecting neurodegeneration in AD.

3.3 | Correlations among CSF BDNF, cognitive performance, and AD biomarkers

Irisin has been reported to promote BDNF expression in the mouse hippocampus.¹⁰ We thus evaluated whether CSF BDNF correlated with irisin, AD biomarkers, and cognition in humans. Results showed a positive correlation between CSF irisin and BDNF (Figure 2A). In line with previous evidence,¹² we found that BDNF was reduced in AD CSF (Table 1). Moreover, BDNF correlated significantly with cognition (Figure 2B) and A β_{42} in the CSF (Figure 2C), but not with tau (Figure 2D). Our findings thus support a correlation among CSF irisin, BDNF, and the extent of memory impairment and AD-related neuropathology in humans.

4 | DISCUSSION

Despite considerable evidence supporting a role of defective brain hormonal signaling in neurodegenerative disorders, notably in AD,^{3,9,13} knowledge is still limited on how such hormones vary in response to disease progression. Here, we focused on investigating CSF levels of irisin, an exercise-induced hormone that is expressed in the brain and was found to promote hippocampal BDNF expression in mice.¹⁰

CSF irisin was significantly associated with cognitive performance, A β_{42} , and BDNF. Importantly, neither irisin nor BDNF correlated with total tau levels in the CSF. This suggests that the decreases in CSF irisin and BDNF in AD are more related to brain amyloid deposition rather than reflecting widespread neurodegeneration, indicated by increased CSF tau.

Irisin and BDNF are two molecular targets previously implicated in AD,^{9,12} and are thought to mediate the brain benefits of physical exercise.^{9,14} Because exercise is increasingly proposed as part of a multimodal strategy to reduce the risk of AD,^{15,16} it is essential to establish potential relationships between exercise-induced mediators and established AD-linked signatures in the CSF. Our current finding that CSF irisin directly correlates with BDNF in humans provides clinical relevance to the previous report that irisin promotes hippocampal BDNF expression in mice.¹⁰ The direct correlations among irisin, BDNF, A β_{42} , and cognitive status here demonstrated further suggest that CSF irisin and BDNF may be useful biomarkers in pharmacological or non-pharmacological interventions in AD.

Limitations of the current study include the reduced size of the study cohort, which may have contributed to the relatively low, albeit statistically significant R² values observed in Pearson correlation analyses, and the cross-sectional approach, which precludes

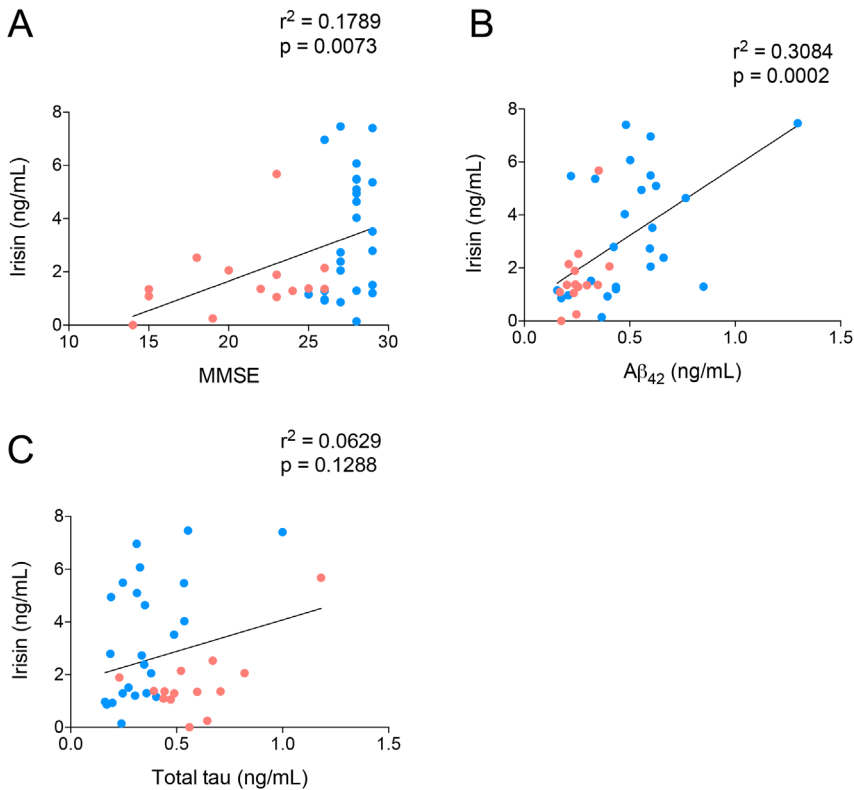


FIGURE 1 Cerebrospinal fluid (CSF) irisin correlates with Mini-Mental State Exam (MMSE) scores and amyloid beta ($A\beta$)₄₂. Correlations between CSF irisin and MMSE scores (A), CSF $A\beta$ ₄₂ (B), or CSF tau (C) in non-demented controls (NDC; blue symbols) and Alzheimer's disease (AD) patients (red symbols). Lines represent Pearson linear regressions (R^2 and P -values as indicated)

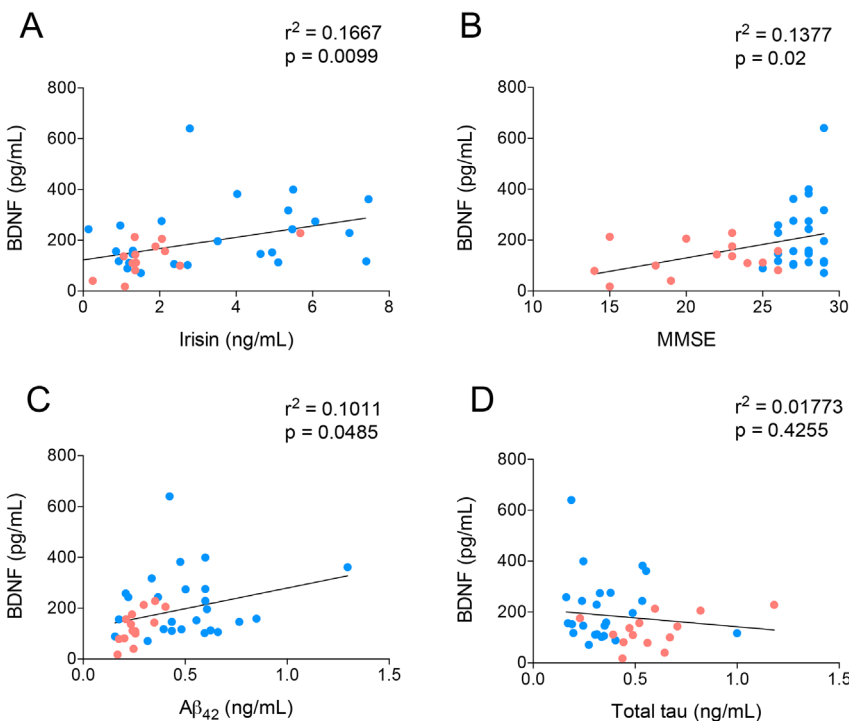


FIGURE 2 Cerebrospinal fluid (CSF) brain-derived neurotrophic factor (BDNF) correlates with irisin, memory, and amyloid beta ($A\beta$)₄₂. Correlations between CSF BDNF and irisin (A), Mini-Mental State Exam (MMSE) scores (B), CSF $A\beta$ ₄₂ (C), or CSF total tau (D) in non-demented controls (NDC; blue symbols) and Alzheimer's disease patients (red symbols). Lines represent Pearson linear regressions, (R^2 and P -values as indicated)

investigation of cognitive trajectories in patients. In addition, the mean age of the AD group was higher than the control group. We note, however, that neither CSF irisin, BDNF, nor $A\beta$ ₄₂ levels significantly correlated with age (not shown), suggesting that the difference in age between groups had a small impact on our findings.

A recent study identified a CSF signature of phosphorylated tau (CSF p-tau) that associates with metabolic, structural, and cognitive changes in dominantly inherited AD.¹⁷ Although neither irisin nor BDNF correlated with total tau in our study, future studies are warranted to determine whether CSF irisin or BDNF correlate with specific p-tau forms in AD patients.

To the best of our knowledge, this is the first report showing that irisin correlates with CSF BDNF and $A\beta_{42}$ and with cognitive status in patients. Irisin is released by muscle tissue upon physical activity and has been shown to regulate metabolism in adipose and other tissues.^{8,18} Irisin recently attracted considerable interest due to its beneficial protective actions in synapses and memory in AD models.⁹ Demonstration that CSF irisin directly correlates with amyloid pathology and cognitive impairment in AD should encourage future studies in larger and diverse cohorts to replicate and extend the current findings, and to explore potential associations with additional markers of AD progression, including markers of synapse damage, CSF p-tau, and imaging data.

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AUTHOR CONTRIBUTIONS

P.M., F.T-M., F.G.D.F, and S.T.F. designed the study. M.V.L., F.C.R., F.K.S., C.D., N.A., and B.V. conducted research. M.V.L., F.C.R, B.V., and S.T.F. analyzed data. M.V.L., P.M., FT-M, F.G.D.F, and S.T.F. discussed results. M.V.L., F.G.D.F, and S.T.F. wrote the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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