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Systemic irisin treatment modulates cognitive function through VEGF-associated hippocampal vascular signaling in chronic cerebral hypoperfusion

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ABSTRACT

Vascular dementia (VaD), primarily caused by chronic cerebral hypoperfusion (CCH), is characterized by progressive cognitive decline associated with neurovascular dysfunction. The present study aimed to investigate whether systemic administration of irisin, an exercise-induced myokine, modulates cognitive performance and VEGF-associated angiogenic signaling in the hippocampus under CCH conditions. Thirty-eight adult male Wistar albino rats were randomly assigned to five groups: control, sham, irisin, ischemia, and ischemia + irisin. CCH was induced via permanent bilateral common carotid artery occlusion. Irisin (100 ng/kg) was administered intraperitoneally three times per week for four weeks. Cognitive performance was assessed using the Morris Water Maze, and VEGF-positive vascular profiles were quantified within a standardized hippocampal area (1 mm^2 per section). CCH resulted in significant impairments in spatial learning and memory, accompanied by a reduction in VEGF-positive vascular profiles in the hippocampus. In healthy rats, irisin administration was associated with improved memory performance and increased VEGF-positive vascular profiles. In ischemic rats, irisin treatment was linked to partial improvements in memory parameters and VEGF-associated vascular changes, although these effects did not reach statistical significance. Learning-phase outcomes were more variable. Notably, the number of VEGF-positive vascular profiles positively correlated with spatial memory performance. These findings suggest that beyond its known neuroprotective properties, irisin may contribute to cognitive support through modulation of angiogenesis-associated signaling under CCH. While further studies are required to clarify optimal dosing strategies and mechanistic pathways, irisin may represent a promising adjunctive candidate for vascular cognitive impairment, particularly in individuals unable to engage in regular physical exercise.

1. Introduction

Vascular dementia (VaD) is the second most common cause of dementia after Alzheimer's disease and represents a major contributor to cognitive decline in the aging population (Kalaria et al., 2008). One of the key mechanisms underlying VaD is chronic cerebral hypoperfusion (CCH); a condition characterized by a persistent reduction in cerebral blood flow caused by vascular insufficiency. Prolonged hypoperfusion disrupts neuronal energy metabolism and leads to progressive impairments in learning and memory (Venkat et al., 2015; Wang et al., 2024). Epidemiological evidence suggests that varying degrees of cerebral hypoperfusion may occur in a large proportion of elderly individuals, highlighting its important role in the development of vascular cognitive impairment (Iadecola, 2013). At the same time; the global number of people living with dementia is expected to nearly double every two decades (Prince et al., 2013). Despite the growing clinical burden of VaD; there is still no approved pharmacological treatment specifically targeting cognitive decline associated with this condition. Therefore; experimental studies aimed at understanding the mechanisms of vascular cognitive impairment and identifying potential therapeutic

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strategies remain of considerable translational importance (Fu et al., 2023). In preclinical research; permanent bilateral common carotid artery occlusion (BCAO) in rodents is widely used as an experimental model of CCH because it reproduces several neuropathological and behavioral features observed in VaD (Stanojlović et al., 2014).

Physical exercise is widely recognized as an effective non-pharmacological intervention for improving outcomes in ischemic brain disorders (MacKay-Lyons et al., 2020). In recent years; increasing attention has been directed toward skeletal muscle as an endocrine organ that releases signaling molecules known as myokines during physical activity. These molecules can act through autocrine; paracrine; or endocrine mechanisms and influence multiple physiological systems (Giudice and Taylor, 2017). Among them; irisin has attracted particular interest. Irisin is an exercise-induced myokine that is released from skeletal muscle during physical activity and is able to cross the blood–brain barrier (BBB). Experimental studies have suggested that irisin may influence several neuroprotective pathways within the central nervous system (Islam et al., 2021; Jodeiri Farshbaf and Alvina, 2021; Ruan et al., 2019). It has therefore been proposed that irisin may contribute, at least in part, to the cognitive benefits associated with regular physical exercise (Wrann, 2015).

In addition to neuroprotective mechanisms, exercise is also known to promote angiogenesis, which may improve tissue perfusion and support functional recovery in ischemic conditions (Tang et al., 2018). Recent studies have shown that irisin can stimulate angiogenic responses in several peripheral ischemic tissues; including bone and cardiac muscle (Kan et al., 2022; Yang et al., 2021). However, whether irisin exerts similar angiogenesis-related effects in the brain under conditions of CCH remains largely unknown.

Enhancing angiogenesis in ischemic tissues remains challenging with conventional pharmacological approaches. Although surgical interventions may improve perfusion in selected cases, they are often not feasible in diffuse vascular diseases. For this reason, exercise-based rehabilitation is commonly recommended for patients with ischemic disorders (Qi et al., 2022). However; many individuals affected by vascular cognitive impairment are elderly and frequently present with comorbidities that limit their ability to engage in regular physical activity. Prolonged hospitalization; advanced age; increased body mass index; and reduced motivation may further restrict mobility in these patients (Chulakadabba et al., 2020). These limitations highlight the need for alternative strategies capable of mimicking the beneficial effects of exercise.

Although the detrimental effects of CCH on hippocampal structure and cognitive function have been extensively documented, therapeutic approaches targeting neurovascular adaptation in this context remain limited. Irisin, an exercise-induced myokine with established neuroprotective properties, has been shown to influence angiogenic processes in peripheral ischemic tissues (Liao et al., 2019; Zhang et al., 2019). However, whether irisin can regulate angiogenesis-associated vascular signaling in the brain under chronic hypoperfusion conditions remains largely unknown. To our knowledge, no previous study has directly examined the relationship between systemic irisin administration, hippocampal VEGF-associated vascular signaling, and spatial memory performance in a chronic cerebral hypoperfusion model. Addressing this gap provides the rationale for the present study.

Therefore, the present study aimed to investigate the effects of systemic irisin administration on cognitive function and VEGF-associated vascular changes in a rat model of CCH. By exploring the relationship between irisin-mediated vascular signaling and spatial memory performance, this study seeks to provide further insight into the potential role of irisin in neurovascular adaptation under hypoperfusive conditions.

2. Materials and methods

2.1. Ethics statement

This study was conducted with the approval of the Balıkesir University Animal Research Centre Local Ethics Committee (approval date: 07.07.2023; approval number: 2023/7–6). All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals and the principles of the 3Rs (Replacement, Reduction, and Refinement).

2.2. Animals

This study was carried out using 38 male Wistar albino rats (10 weeks old, 250–300 g) obtained from the Experimental Animal Production, Care and Research Center of Balıkesir University. The animals were housed in standard cages with free access to water and laboratory chow. They were maintained under a 12 h light/12 h dark cycle at a constant room temperature of 23 ± 2 °C and relative humidity of 60%. All procedures and reporting followed the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

2.3. Experimental design

The rats were randomly divided into 5 groups: (1) Control ($n = 7$), (2) sham ($n = 8$), (3) ischemia (n = 8), (4) irisin (n = 7), (5) ischemia + irisin (n = 8). Considering that the mortality rate following BCAO surgery has been reported to be approximately 25% within the first 14 postoperative days, the number of animals allocated to the surgical groups was slightly higher (Wang et al., 2020). Sample size adequacy was evaluated a priori using G*Power 3.1 (Heinrich Heine University; Düsseldorf; Germany). A one-way ANOVA (fixed effects; omnibus test) with five groups; an alpha level of 0.05; and an effect size 0.65 was assumed. The effect size (f = 0.65) is consistent with standard practice in rodent experimental studies; where medium-to-large effect sizes are commonly used for power analyses (Erdoğan, 2024). Under these assumptions, the minimum required total sample size to achieve a statistical power of 0.80 was calculated as 35 animals (approximately seven animals per group). Therefore, despite the mortality observed in the ischemia groups, the final sample size remained within the range required to maintain adequate statistical power.

No experimental procedure was performed in the control group. The sham group underwent bilateral exposure of the common carotid arteries without occlusion. The ischemia groups underwent BCAO. Irisin treatment was administered exclusively to the irisin and ischemia + irisin groups at a dose of 100 ng/kg via intraperitoneal injection. The control, sham, and ischemia-only groups did not receive irisin. A graphical timeline illustrating the experimental schedule is provided in Supplementary Fig. S1.

2.4. BCAO model

Chronic cerebral hypoperfusion was induced by BCAO under anesthesia (80 mg/kg ketamine and 8 mg/kg xylazine, intraperitoneally) (Cao et al., 2018). After shaving the ventral neck region, the common carotid arteries were exposed bilaterally through a midventral cervical incision and carefully separated from surrounding connective tissues and the vagus nerve. Permanent double ligation was performed using 4–0 silk sutures to achieve complete occlusion without subsequent reperfusion. The arteries were then transected between the ligation points to prevent recanalization (Supplementary Fig. S2). The incision was sutured and the animals were returned to their cages until recovery from anesthesia. The entire surgical procedure lasted approximately 20 min per animal. In the sham group, the carotid arteries were exposed in the same manner but no ligation was performed.

2.5. Irisin administration

Beginning one day after surgery, recombinant irisin (r-irisin; #11451, Cayman Chemicals, Ann Arbor, MI, USA) was administered intraperitoneally. A 100 μg recombinant irisin vial was diluted with deionized water and injected in a volume of 0.1 ml at a final dose of 100 ng/kg. Irisin was administered three times per week (Monday, Wednesday, and Friday) for four weeks. The dose and intermittent administration schedule were selected based on previous studies demonstrating biological efficacy with similar systemic protocols (Guo et al., 2023; Shahabi et al., 2024; Yardimci et al., 2023). Intermittent dosing was also chosen to minimize handling stress associated with frequent injections (Gouveia and Hurst, 2017). Animals in the sham and ischemia groups received an equivalent volume of physiological saline according to the same schedule.

2.6. Locomotor activity

After the final irisin injection, locomotor activity was evaluated using an open field (OF) test apparatus. The apparatus consisted of a black square arena measuring 1 × 1 m, surrounded by walls 50 cm in height. Each rat was placed in the center of the arena and spontaneous locomotor activity was recorded for 5 min using the Noldus EthoVision XT video tracking system.

All behavioral assessments were performed by a researcher blinded to the treatment groups.

2.7. The Learning-Memory Test

On the day following the OF test, spatial learning and memory were assessed using the Morris Water Maze (MWM) (Morris, 1984). The apparatus consisted of a circular Plexiglas pool (140 cm diameter, 75 cm height) filled with water to a depth of 50 cm. A hidden platform was positioned 1 cm below the water surface. Each rat underwent testing for four consecutive days during the acquisition phase. On the fifth day, the platform was removed and a probe trial was performed. Escape latency and time spent in each quadrant were recorded and analyzed using the Noldus EthoVision XT tracking system.

Behavioral assessments were conducted by a researcher blinded to treatment allocation.

2.8. Sacrifice and tissue collection

One day after completion of the behavioral experiments, the rats were sacrificed by decapitation under anesthesia. Whole brain tissues were carefully removed and fixed in formaldehyde for subsequent immunohistochemical analysis.

2.9. Immunohistochemistry

Brain tissue samples were fixed in 10% neutral-buffered formalin, processed routinely, and embedded in paraffin. Hippocampal sections were obtained in the coronal plane, focusing on the CA1 pyramidal layer and the adjacent stratum oriens and stratum radiatum, regions known to be particularly sensitive to ischemic injury (Schmidt-Kastner, 2015). Sections of 4 μm thickness were prepared and stained with hematoxylin and eosin for routine histopathological evaluation. Additional 4 μm sections were mounted on positively charged glass slides for immunohistochemical analysis.

Antigen retrieval was performed using CC1 solution (pH 8.0) (Ref. 950-CC124, Ventana Medical Systems, Tucson, AZ, USA). Sections were incubated with primary human monoclonal anti-VEGF antibody (JH121; dilution 1:150; sc-57,496, Santa Cruz Biotechnology, Texas, USA) for 32 min at 37 °C. Visualization was achieved using the OptiView DAB IHC Detection Kit followed by the OptiView Amplification Kit (Ventana), each applied for 12 min. All staining procedures were performed on the VENTANA BenchMark Ultra automated staining platform according to the manufacturer's protocol.

VEGF is a key regulator of angiogenesis and vascular remodeling (Ferrara, 2004). In the present study, VEGF immunoreactivity was evaluated as an indicator of angiogenesis-associated signaling activity within vascular structures rather than as an endothelial cell–specific marker of absolute microvascular density. VEGF immunoreactivity was identified as brown cytoplasmic staining within vascular endothelial profiles. Quantification was restricted to VEGF-positive structures displaying clear vascular morphology. Only VEGF-immunoreactive profiles showing identifiable vascular characteristics, such as lumen-like structures and vessel-like organization, were included in the analysis. Non-vascular VEGF staining in neural or glial cells was not considered during quantification.

Three coronal brain sections per animal, obtained at 150 μm intervals, were analyzed. VEGF-positive vascular profiles were quantified in 10 non-overlapping fields at ×200 magnification within a defined hippocampal area of 1 mm^2. The mean number of VEGF-positive vascular profiles within the predefined 1 mm^2 sampling area was calculated for each animal. This standardized sampling ensured anatomical and area consistency across all groups.

All immunohistochemical assessments were performed by a researcher blinded to treatment allocation.

2.10. Statistical analysis

All statistical analyses were performed using SPSS software for Windows, version 25.0 (SPSS, Chicago, IL, USA). For normally distributed data, group differences were evaluated using one-way ANOVA followed by Tukey's post hoc test. Non-parametric data were analyzed using the Kruskal–Wallis test. Learning performance across the four-day acquisition period of the MWM was analyzed using GLM repeated measures ANOVA. Correlations between variables were assessed using Spearman correlation analysis. A value of $P < 0.05$ was considered statistically significant.

3. Results

On the day of surgery, four rats in the ischemia groups died, and one additional rat died on the following day. To maintain consistent group sizes, surgery was repeated for these five animals on the next day. The overall mortality rate in the ischemia groups was 31%.

3.1. Locomotor activity

Locomotor activity was assessed using the OF test. The mean total distance traveled was 1517.82 ± 340.35 cm in the control group, 1881.15 ± 625.07 cm in the sham group, 1779.64 ± 550.89 cm in the ischemia group, 1866.27 ± 310.01 cm in the irisin group, and 1891.93 ± 387.75 cm in the ischemia + irisin group.

The mean velocity values were 5.06 ± 1.14 cm/s, 6.27 ± 2.08 cm/s, 5.93 ± 1.84 cm/s, 6.22 ± 1.03 cm/s, and 6.31 ± 1.30 cm/s for the control, sham, ischemia, irisin, and ischemia + irisin groups, respectively. No statistically significant differences were observed among the groups for either parameter measured in the OF test (distance traveled: $F(4,33) = 0.8148$, $P = 0.5249$; velocity: $F(4,33) = 0.9872$, $P = 0.4281$) (Fig. 1A–B).

These results indicate that locomotor activity did not differ significantly among the experimental groups.

3.2. Learning

Spatial learning performance was assessed using the MWM during the four-day acquisition phase.

On day 1, the escape latencies were 50.43 ± 12.42 s for the control group, 54.02 ± 10.12 s for the sham group, 52.38 ± 8.98 s for the

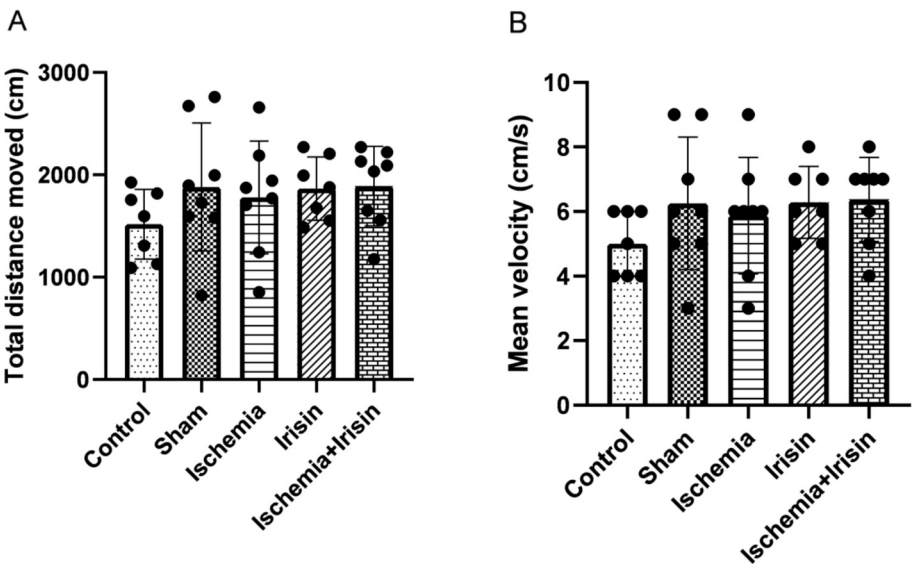

Fig. 1. Chronic cerebral hypoperfusion did not affect locomotor activity in the open field test. A – Total distance moved (cm). B – Mean velocities of the groups (cm/s). No statistically significant differences were observed among the groups. (Significance level is $P < 0.05$).

ischemia group, 47.71 ± 9.84 s for the irisin group, and 55.96 ± 7.48 s for the ischemia + irisin group. No statistically significant differences were observed among the groups on the first day ($F(4,33) = 0.7908$, $P = 0.5396$) (Fig. 2A).

On day 2, a significant difference among the groups was detected ($F(4,33) = 6.662$, $P = 0.0005$). The mean escape latencies were 40.57 ± 14.88 s in the control group, 42.25 ± 12.67 s in the sham group, 54.00 ± 8.40 s in the ischemia group, 24.43 ± 13.05 s in the irisin group, and 50.25 ± 10.67 s in the ischemia + irisin group. Post hoc analysis revealed that the irisin group had significantly shorter escape latencies compared with both the ischemia group ($P = 0.0003$) and the ischemia + irisin group ($P = 0.0019$). Although the irisin group also showed lower escape latencies than the sham group, this difference did not reach statistical significance ($P = 0.052$) (Fig. 2B).

On day 3, the mean escape latencies of the ischemia and ischemia + irisin groups were significantly higher than those of the sham group ($P = 0.0366$ and $P = 0.0087$, respectively) and the irisin group ($P = 0.009$ and $P = 0.002$, respectively). The mean values were 36.43 ± 16.66 s in the control group, 32.63 ± 7.15 s in the sham group, 50.38 ± 12.61 s in the ischemia group, 28.57 ± 7.41 s in the irisin group, and 53.75 ± 12.53 s in the ischemia + irisin group ($F(4,33) = 6.847$, $P = 0.0004$) (Fig. 2C).

On day 4, the mean escape latencies were 33.57 ± 10.64 s in the control group, 27.25 ± 17.16 s in the sham group, 45.50 ± 15.84 s in the ischemia group, 27.29 ± 11.01 s in the irisin group, and 46.63 ± 14.72 s in the ischemia + irisin group. No statistically significant pairwise differences were detected among the groups on this day ($F(4,33) = 3.434$, $P = 0.0188$) (Fig. 2D). Notably, 50% of the rats in both the ischemia and ischemia + irisin groups failed to locate the hidden platform on day 4, whereas nearly all animals in the control, sham, and irisin groups successfully reached the platform.

Within-group comparisons revealed a significant reduction in escape latency from day 1 to day 3 in the sham group ($F(3,21) = 6.846$, $P = 0.002$; day 1 vs day 3, $P = 0.025$). Similarly, the irisin group showed significantly lower escape latencies on subsequent testing days compared with day 1 ($F(3,18) = 9.329$, $P = 0.001$; $P = 0.013$, $P = 0.024$, and $P = 0.040$ for days 2–4, respectively) (Fig. 2E).

In the MWM, ischemia resulted in significant impairments in spatial learning, as reflected in prolonged escape times on days 2 and 3. The ischemia + irisin group did not show statistically significant improvements in learning performance compared to the ischemia group. Although escape times were shorter in the irisin-treated ischemic group compared to the ischemia group in the 2nd day, this improvement was not statistically significant.

3.3. Memory

Spatial memory performance was evaluated during the probe trial of the MWM.

A significant difference among the groups was observed in the time spent in the target quadrant ($F(4,33) = 5.907$, $P = 0.0011$). The mean durations were 10.57 ± 2.04 s in the control group, 9.53 ± 1.82 s in the sham group, 7.78 ± 1.54 s in the ischemia group, 11.91 ± 1.90 s in the irisin group, and 9.25 ± 1.35 s in the ischemia + irisin group. The ischemia group spent significantly less time in the target quadrant compared with both the control ($P = 0.0291$) and irisin ($P = 0.0005$) groups. A significant difference was also detected between the irisin and ischemia + irisin groups ($P = 0.0412$). Although irisin administration in ischemic rats increased the time spent in the target quadrant, the difference between the ischemia and ischemia + irisin groups did not reach statistical significance. On the other hand, no statistically significant difference was detected between the control and ischemia + irisin groups ($P = 0.587$), which may indicate a favorable trend toward improvement in learning performance in the ischemia + irisin group (Fig. 3A).

The number of entries into the target quadrant also differed among the groups. The mean entry numbers were 6.43 ± 1.51 in the control group, 6.13 ± 2.03 in the sham group, 3.63 ± 0.92 in the ischemia group, 5.43 ± 1.40 in the irisin group, and 4.38 ± 1.30 in the ischemia + irisin group. A significant difference was detected only between the control and ischemia groups ($P = 0.0141$). No statistically significant difference was observed between the control and ischemia + irisin groups ($P = 0.217$). These results may also indicate a tendency of partial improvement following irisin treatment. (Fig. 3B).

A significant difference among the groups was also observed in the time spent in the opposite quadrant ($F(4,33) = 4.173$, $P = 0.0076$). The mean durations were 10.43 ± 3.59 s for the control group, 9.78 ± 1.86 s for the sham group, 10.85 ± 2.66 s for the ischemia group, 6.29 ± 1.48 s for the irisin group, and 10.63 ± 2.36 s for the ischemia + irisin group. The irisin group spent significantly less time in the opposite quadrant compared with the control group ($P = 0.0284$). In addition, both the ischemia and ischemia + irisin groups spent significantly more time in the opposite quadrant than the irisin group ($P = 0.0096$ and $P = 0.0151$, respectively). Although irisin treatment reduced the mean time spent in

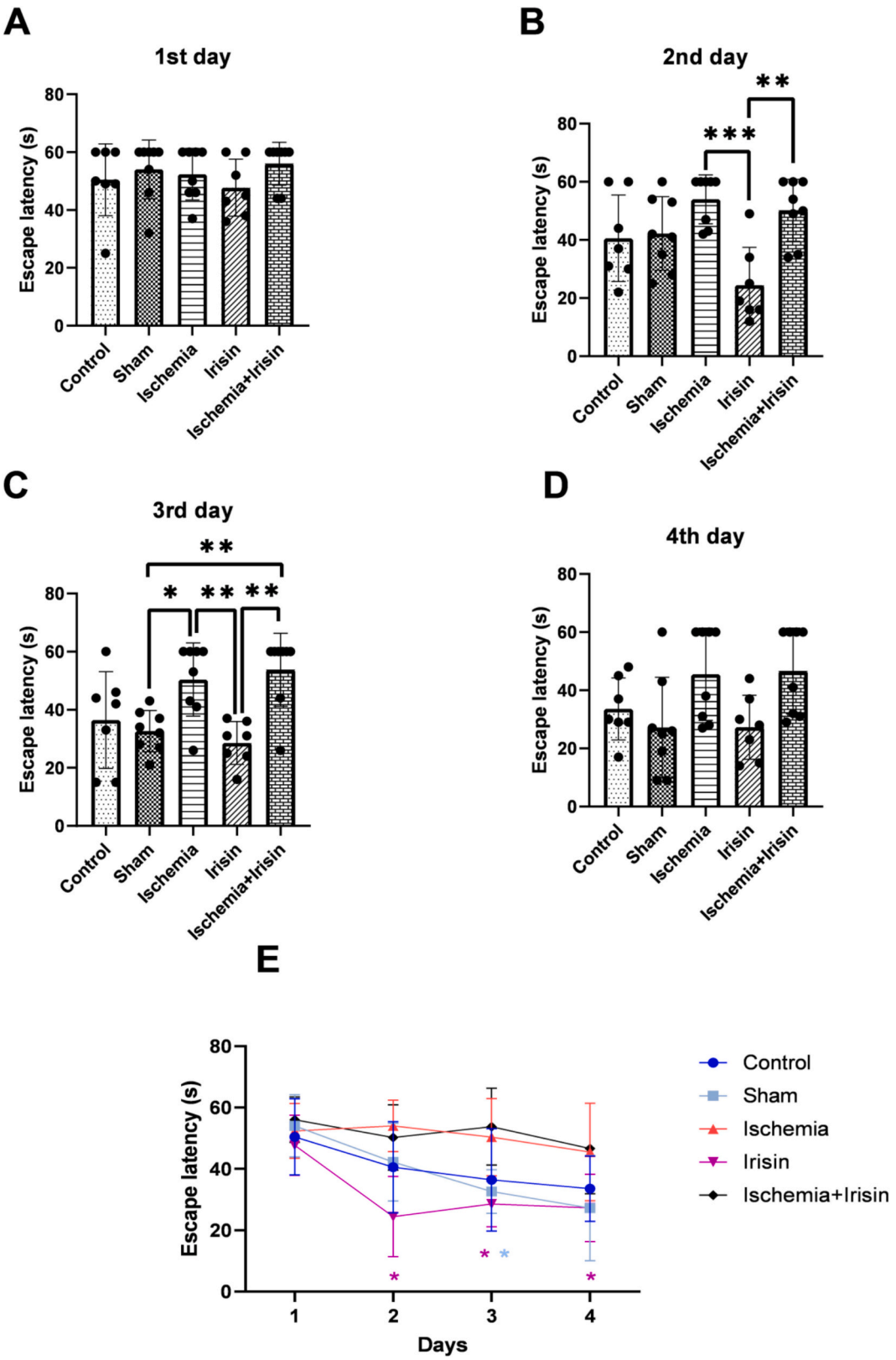

Fig. 2. Effects of chronic cerebral hypoperfusion and irisin treatment on spatial learning in the Morris Water Maze A – Escape latency to reach the hidden platform on Day 1. B – Escape latency on Day 2. C – Escape latency on Day 3. D – Escape latency on Day 4. E – Mean escape latency across the four consecutive training days. Data are expressed as mean ± SD.*P < 0.05, **$P < 0.01$, ***$P < 0.001$ compared with Day 1.

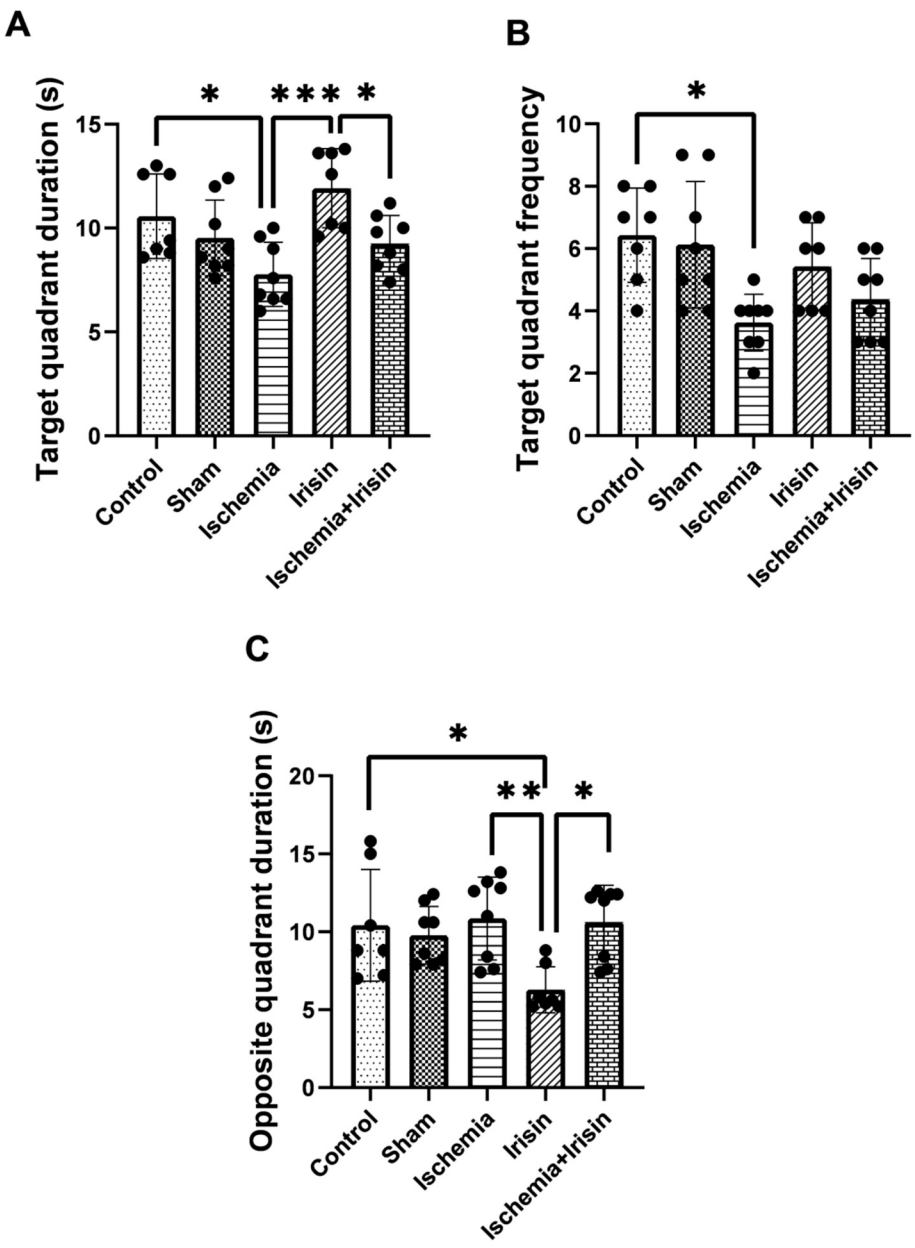

Fig. 3. Effects of chronic cerebral hypoperfusion and irisin treatment on spatial memory performance in the Morris Water Maze probe trial. A – Time spent in the target quadrant (s). B – Number of entries into the target quadrant. C – Time spent in the opposite quadrant (s). *P < 0.05, **P < 0.01, ***$P < 0.001$.

the opposite quadrant in ischemic rats, this difference did not reach statistical significance (Fig. 3C).

No significant differences were observed among the groups in the number of entries into the opposite quadrant ($P = 0.414$). The mean entry numbers were 5.29 ± 2.43 in the control group, 3.25 ± 1.75 in the sham group, 3.75 ± 1.04 in the ischemia group, 3.86 ± 1.46 in the irisin group, and 4.13 ± 1.55 in the ischemia + irisin group.

3.4. Immunohistochemistry

All VEGF-positive vascular profile counts were obtained using a predefined standardized sampling frame (1 mm^2 per section) to ensure area consistency across groups.

The mean numbers of VEGF-positive vascular profiles were 9.17 ± 1.17 in the control group, 7.50 ± 0.55 in the sham group, 4.33 ± 0.52 in the ischemia group, 11.83 ± 1.17 in the irisin group, and 8.00 ± 0.89 in the ischemia + irisin group. The ischemia group showed a significantly lower number of VEGF-positive vascular profiles compared with both the control ($P = 0.0103$) and irisin ($P = 0.0001$) groups. In addition, the irisin group exhibited a higher number of VEGF-positive vascular profiles than the sham group ($P = 0.0254$).

Although the difference between the ischemia and ischemia + irisin groups did not reach statistical significance, the ischemia + irisin group showed higher VEGF-positive vascular profile counts compared with the ischemia-only group. Furthermore, while the ischemia group displayed significantly lower VEGF-positive vascular profile counts than the control group, no significant difference was observed between the ischemia + irisin and control groups. Representative immunohistochemical images of VEGF staining in the hippocampus are presented in Fig. 4–6.

These findings may indicate a trend toward partial amelioration of VEGF-associated vascular changes following irisin administration in ischemic rats.

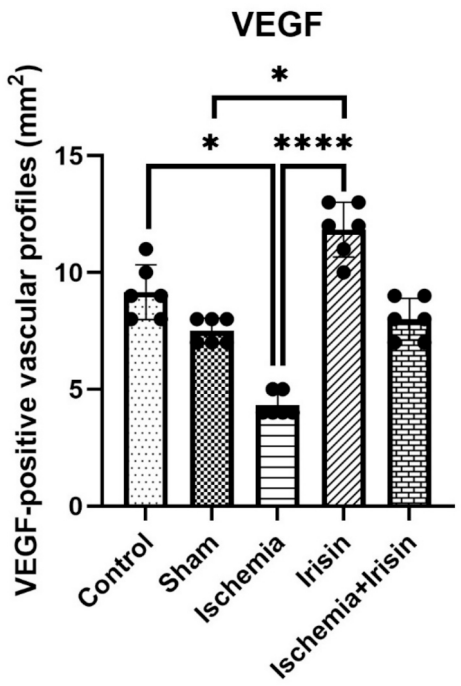

Fig. 4. Quantification of VEGF-positive vascular profiles in the hippocampus across experimental groups. VEGF-positive vascular profiles were quantified within a standardized hippocampal area (1 mm^2). Data are presented as mean ± SD with individual data points.*P < 0.05, ****$P < 0.0001$.

3.5. Correlation analyses

Correlation analysis revealed a significant positive association between the number of VEGF-positive vascular profiles and time spent in the target quadrant during the probe trial ($r = 0.631$, P = 0.0001) (Fig. 7A).

In contrast, a significant negative correlation was observed between VEGF-positive vascular profiles and time spent in the opposite quadrant ($r = -0.380$, $P = 0.038$) (Fig. 7B).

Correlations between VEGF-positive vascular profiles and memory performance further support a functional link between angiogenic signaling and cognitive outcomes.

4. Discussion

In the present study, CCH resulted in significant reductions in VEGF-positive vascular profiles in the hippocampus and was accompanied by impairments in spatial memory. Although the detrimental effects of BCAO on hippocampal function have been well documented, the present findings extend the existing literature by providing a proof-of-concept that systemic irisin administration may modulate VEGF-associated angiogenic signaling in the hippocampus and relate these vascular changes to spatial memory performance under CCH conditions.

It should also be noted that CCH did not produce any detectable changes in locomotor activity, as indicated by the OF test results. This observation is consistent with previous studies reporting preserved locomotor performance in similar models of CCH (Cechetti et al., 2012; Damodaran et al., 2014). Therefore, the impairments observed in the MWM are unlikely to be related to motor deficits and more likely reflect alterations in cognitive function.

In the present study, irisin administration was associated with a modest but noteworthy tendency toward improved cognitive performance following CCH. Although not all behavioral parameters reached statistical significance, ischemic rats treated with irisin showed a general trend toward better task acquisition and slightly improved memory retrieval compared with untreated ischemic animals. For example, irisin-treated rats tended to spend more time in the target quadrant and displayed more normalized entry frequencies during the probe trial, suggesting a potential beneficial influence of irisin on hippocampus-dependent memory processes.

BCAO markedly reduces cerebral blood flow, and compensatory circulation through the vertebral arteries remains insufficient during the early postoperative period (Wang et al., 2020). As a result; CCH disrupts neuronal energy metabolism; particularly in brain regions that are highly vulnerable to oxygen deprivation. Reduced glucose utilization can trigger excitotoxicity; mitochondrial dysfunction; and ultimately neuronal death (Rajeev et al., 2022; Wang et al., 2020). Breakdown of the BBB and subsequent neuroinflammatory cascades further contribute to neuropathological alterations associated with cognitive decline,

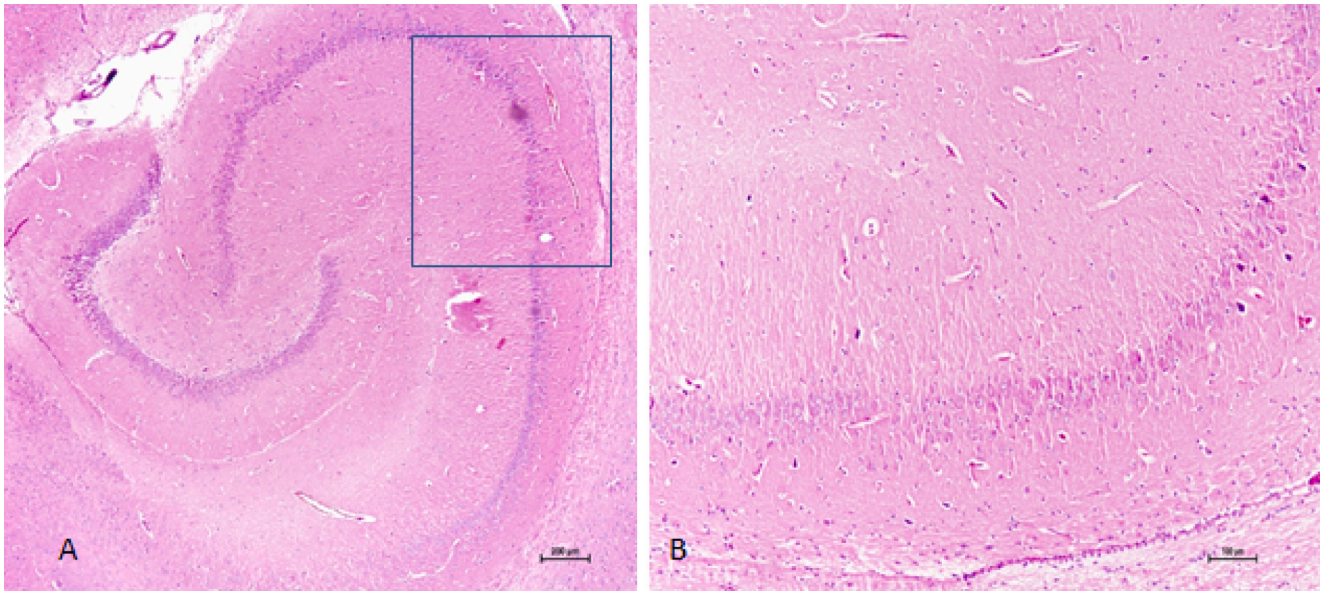

Fig. 5. Representative hematoxylin and eosin (H&E)–stained sections of the hippocampus indicating the region used for immunohistochemical analysis. A – Low-magnification view of the hippocampus, 40×, B – Higher-magnification view of the CA1 region corresponding to the boxed area in panel A, 100×. Scale bars: A = 200 μm; B = 100 μm.

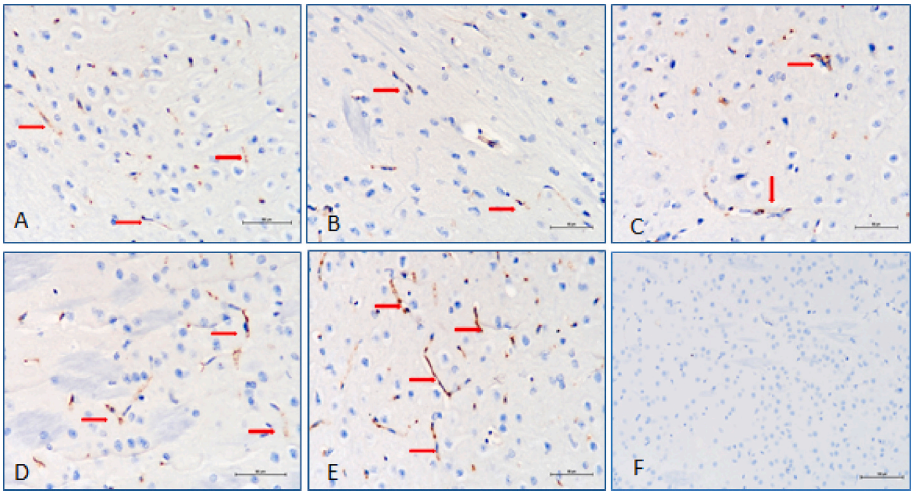

Fig. 6. Representative immunohistochemical staining for VEGF in hippocampal sections across experimental groups. A – Control group. B – Sham group. C – Ischemia group. D – Irisin group. E – Ischemia + irisin group. F – Negative control. Red arrows indicate VEGF-immunoreactive vascular profiles displaying vessel-like morphology. Only VEGF-positive structures with clear vascular morphology were included in the quantitative analysis. Magnification: A–E, ×200; F, ×100. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

including glial activation and neuronal loss (Rajeev et al., 2022). Because the hippocampus is particularly sensitive to ischemic injury; these processes can impair hippocampus-dependent cognitive functions (Lee et al., 2021). Consistent with these mechanisms and previous reports, CCH in our study resulted in significant impairments in spatial learning and memory.

One possible explanation for the modest magnitude of the observed effects is the relatively short treatment duration used in the present study. In a recent study investigating the systemic effects of irisin on brain monoamine levels, irisin was administered for 10 weeks and significant neurochemical changes were reported (Yardimci et al., 2023). Although the dose used in that study (100 ng/kg) was similar to ours; the treatment duration was considerably longer. In another study using a mouse model of middle cerebral artery occlusion; a single bolus injection of irisin (0.2 μg/g) significantly reduced infarct volume (Li et al., 2017). Furthermore; Wang and Pan (2016) reported that systemic irisin administration reduced depressive-like behaviors in rats at doses of 100 ng/kg and above; indicating that this dose range is biologically active in the central nervous system. In the BCAO model; cerebral blood flow remains only partially restored during the first four weeks following surgery; and full recovery may require up to eight weeks (Otori et al., 2003). Therefore, it is possible that the four-week treatment period used in the present study was not sufficient to fully reveal the potential cognitive benefits of irisin. Longer administration periods may allow a clearer manifestation of its neurovascular and behavioral effects. These findings suggest that the cognitive effects of irisin may emerge gradually as neurovascular adaptations develop over time.

Although irisin showed a tendency toward improved spatial memory performance, the longer platform-finding times observed in some learning parameters in the ischemia + irisin group compared with the ischemia-only group suggest that its effects during the learning phase may be more variable. This variability may reflect differences in treatment timing, dosage, or the differential sensitivity of distinct cognitive processes to irisin. It is also possible that irisin exerts a stronger influence on memory consolidation than on the initial acquisition of spatial information. Similar dissociations between learning and memory processes have been reported previously. For example, amphetamine has been shown to enhance memory consolidation in rats without consistently improving initial learning performance, suggesting that these cognitive phases may rely on partially distinct neurobiological mechanisms (Simon and Setlow, 2006).

Irisin has been shown to cross the BBB and activate several neuroprotective pathways, including increased BDNF expression, enhanced synaptogenesis, and improved neuronal survival in the hippocampus (Jodeiri Farshbaf and Alvina, 2021). Consistent with these mechanisms; rats in the irisin-treated group in our study showed better behavioral performance in the MWM. Specifically; the irisin group learned the location of the hidden platform more rapidly; spent the highest mean time in the target quadrant during the probe trial; and spent the least time in the opposite quadrant. These findings suggest that irisin may exert cognitive-enhancing effects even under non-ischemic conditions. Supporting this possibility; previous human studies have reported positive associations between circulating irisin levels and cognitive performance in healthy individuals (Kuster et al., 2017; Tsai and Pai, 2021).

The immunohistochemical findings of the present study showed that CCH reduced the number of VEGF-positive vascular profiles in the hippocampus. In addition, irisin administration was associated with a mild tendency toward improvement in VEGF-associated vascular changes in both healthy and ischemic rats. Previous studies have demonstrated that the BCAO model leads to capillary rarefaction in the brain and reduced microvascular cerebral blood flow (Leardini-Tristao et al., 2017). Similarly; reductions in capillary density; particularly within white matter regions; have been consistently reported in studies of VaD and CCH (Lee et al., 2019; Shao et al., 2010).

Although VEGF can be expressed by multiple neural cell types under ischemic conditions, previous studies in CCH models have demonstrated predominant localization of VEGF protein to vascular endothelial cells, accompanied by parallel upregulation of VEGF receptors (Hai et al., 2003). This pattern supports the notion that VEGF participates in endothelial autocrine and paracrine signaling mechanisms during hypoperfusion-induced vascular adaptation. Therefore, while VEGF immunostaining does not represent an endothelial cell–specific structural marker of absolute capillary density, the VEGF-positive vascular profiles quantified in the present study likely reflect angiogenesis-associated signaling activity within the hippocampal microvasculature.

Exercise training is known to promote angiogenesis via increasing VEGF in cerebral vessels in a number of brain areas including the hippocampus (Pahlavani, 2023). Irisin; an exercise-induced myokine; has been proposed as a mediator of several beneficial effects of physical activity on the brain. An increasing body of evidence indicates that irisin exerts cerebroprotective effects through multiple mechanisms; including reduction of oxidative stress; attenuation of neuroinflammation; protection of the BBB; and regulation of neuronal survival

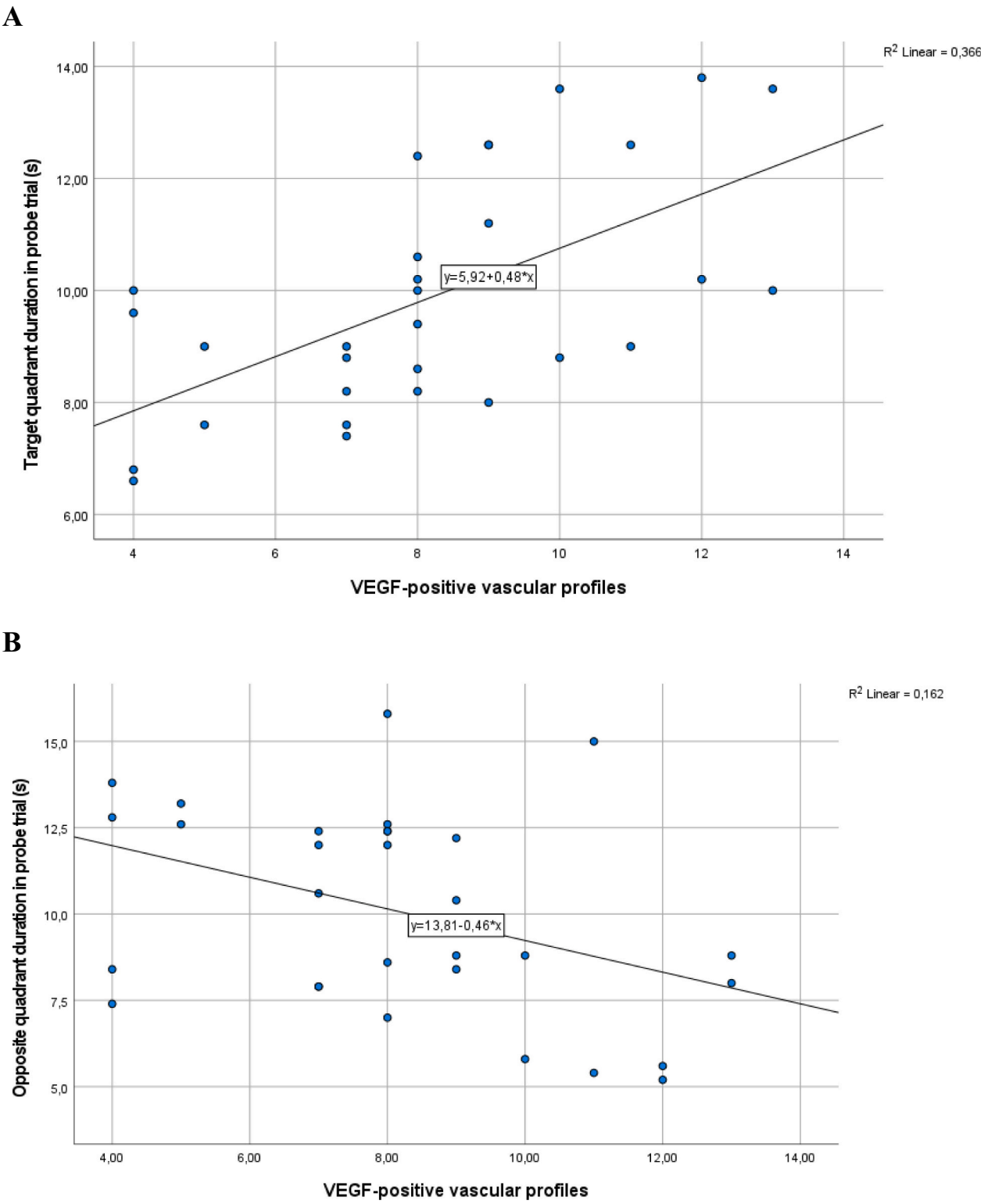

Fig. 7. Correlation between VEGF-positive vascular profiles in the hippocampus and spatial memory performance in the Morris Water Maze probe trial. A – Correlation between the number of VEGF-positive vascular profiles (per mm^2) and time spent in the target quadrant. B – Correlation between the number of VEGF-positive vascular profiles (per mm^2) and time spent in the opposite quadrant.

and stem cell proliferation (Liu et al., 2024). However; with the exception of some evidence on bone fracture healing and myocardial ischemia; we have not encountered research in the literature that directly investigates the angiogenesis-stimulating effect of irisin in the brain (Kan et al., 2022; Liao et al., 2019; Oranger et al., 2023; Yang et al., 2021). Therefore, beyond its well-established neuroprotective properties, the present findings suggest that irisin may exert beneficial effects in the ischemic brain partly through modulation of VEGF-associated angiogenic signaling, pointing to a potential additional mechanism underlying its neurovascular actions. This observation is particularly relevant because it suggests a previously underexplored link between an exercise-induced myokine and angiogenesis-associated

vascular signaling in a CCH model.

In a recent study, electroacupuncture was shown to facilitate vascular remodeling and reduce infarct size after partial cerebral ischemia through activation of the irisin-mediated VEGF/Akt/eNOS signaling pathway (Cao et al., 2025). Although the experimental conditions in that study—including the ischemia model and evaluation time—differ from those used in the present work, the findings provide indirect support for a role of irisin in regulating angiogenesis-related vascular responses in the ischemic brain. In the present study, four weeks of systemic irisin administration was associated with an increase in VEGF-positive vascular profiles in the hippocampus of healthy rats. Moreover, although the difference between the ischemia and ischemia + irisin groups did not reach statistical significance, the number of VEGF-positive vascular profiles in the ischemia + irisin group was comparable to control levels, in contrast to the marked reduction observed in the ischemia-only group. Taken together, these findings suggest that the potential cognitive-enhancing effects of irisin may be partially mediated through modulation of VEGF-associated angiogenic signaling within the hippocampus. This interpretation is further supported by the significant correlations observed between VEGF-positive vascular profiles and spatial memory performance in our study.

VEGF has previously been shown to improve spatial learning and memory performance in rodents (Pati et al., 2009). More recently; VEGF-loaded nanofiber delivery systems have been proposed as a potential therapeutic approach to alleviate cognitive deficits associated with CCH by promoting neurovascular repair and reducing neuronal apoptosis and synaptic damage (Wu et al., 2023). These findings highlight the therapeutic potential of VEGF-mediated neurovascular mechanisms in vascular cognitive impairment. However, such approaches generally require invasive delivery methods. In contrast, our findings suggest that a systemically administered agent such as irisin may influence VEGF-associated vascular signaling and contribute to the mitigation of cognitive deterioration without the need for surgical intervention or exercise-based rehabilitation. This possibility may be particularly relevant for patients with VaD who are unable to participate in regular physical exercise.

Hippocampal activity has been associated with increased endothelial cell formation, and adult neurogenesis is known to occur within an angiogenic niche (Palmer et al., 2000). VEGF; a hypoxia-inducible protein that promotes angiogenesis; is primarily localized to vascular endothelial cells in hypoperfused brain tissue and has been widely used to identify angiogenesis-related vascular responses (Hai et al., 2003). Consistent evidence indicates a close relationship between vascular density and cognitive performance (Clark et al., 2009). Interestingly; studies in healthy adult rats have suggested that learning and memory performance may depend more strongly on angiogenesis than on neurogenesis (Kerr et al., 2010). Therefore, stimulating neovascularization seems to be the main factor to enhance cognitive functions.

In the present study, the number of VEGF-positive vascular profiles showed a strong positive correlation with time spent in the target quadrant and a negative correlation with time spent in the opposite quadrant during the probe trial. These findings support an association between angiogenesis-related vascular signaling and spatial memory performance and suggest that irisin's potential cognitive-enhancing effects may be partly mediated through modulation of VEGF-associated angiogenic signaling in the hippocampus. Although irisin administration was associated with a tendency to increase VEGF-positive vascular profiles in ischemic rats, the absence of statistically significant improvements in memory performance between the ischemia and ischemia + irisin groups may indicate that angiogenic signaling had not yet translated into fully functional vascular remodeling within the four-week treatment period. Newly formed capillaries require time to mature, stabilize, and integrate into the existing vascular network to provide sufficient metabolic support for neuronal activity (Hayashi et al., 2003). Moreover; improvements in cognitive performance may depend not only on vascular density but also on restoration of neurovascular coupling and synaptic plasticity; processes that may develop more slowly than the initial angiogenic response (Yang and Torbey, 2020). Therefore, longer treatment durations or delayed behavioral assessments may be necessary to fully capture the functional consequences of irisin-associated angiogenic signaling.

Our study has several limitations that should be considered when interpreting the findings. First, the present study did not directly assess the BBB permeability of irisin. Nevertheless, existing evidence suggests that systemically administered irisin can reach the central nervous system. Previous studies have demonstrated that irisin is capable of crossing the BBB in mice, and BBB permeability characteristics in mice are considered comparable to those in rats (Islam et al., 2021; Murakami et al., 2000). In addition, irisin has been proposed as a mediator of exercise-induced neuroprotection and has been associated with improvements in motor and cognitive functions in several neurological disease models (Caproni et al., 2025). Therefore, although our findings indicate potentially beneficial effects of irisin on hippocampus-dependent cognitive functions under ischemic conditions, the precise mechanisms by which irisin influences the central nervous system require further investigation.

Second, only male rats were included in the present study. Sex hormones are known to influence cerebral blood flow regulation, neuroinflammatory responses, angiogenesis, and susceptibility to ischemic injury, potentially leading to sex-dependent differences in neurovascular outcomes (Roof et al., 1993; Roof and Hall, 2000). Epidemiological studies have also reported sex-related differences in the incidence of VaD, with several reports suggesting a higher prevalence in men (Di Carlo et al., 2002; Ruitenberg et al., 2001). Considering this clinical pattern, male rats were selected in the present study in order to model vascular cognitive impairment in a population that may be more vulnerable to the disease. Nevertheless, sex is increasingly recognized as an important biological variable in preclinical research, and future studies including female animals with appropriate monitoring of hormonal cycles will be necessary to determine whether irisin-mediated neurovascular and cognitive effects differ between sexes.

Another limitation relates to the age of the experimental animals. CCH and vascular cognitive impairment predominantly affect aged populations, and the aged brain may respond differently to ischemic insults and neurovascular interventions compared with young adult animals. In the present study, 10-week-old rats were used to provide a homogeneous baseline and to reduce surgical complications and mortality associated with BCAO in aged animals, which is a common approach in preclinical neurovascular research (Candelario-Jalil and Paul, 2021; Macrae, 2011).

In addition, the present study did not include biochemical quantification of circulating or hippocampal irisin levels. Future studies combining pharmacokinetic measurements with molecular pathway analyses may help clarify dose–response relationships and the signaling mechanisms underlying irisin-mediated neurovascular effects.

Finally, VEGF immunostaining was used to evaluate angiogenesis-related activity in the hippocampus. Although VEGF is a well-established regulator of angiogenesis (Ferrara, 2004); it is not exclusively expressed by endothelial cells and therefore cannot be considered a strictly endothelial-specific marker of absolute microvascular density. The inclusion of additional endothelial markers; such as CD31 or von Willebrand factor; would allow a more comprehensive characterization of vascular remodeling. However; CD31 expression may also increase during inflammatory responses; which can complicate the distinction between inflammation-associated endothelial activation and true angiogenesis (Leung and Jensen, 2013). Taken together, VEGF immunostaining in the present study should be interpreted as a functional proxy for angiogenesis-related signaling in the hippocampal microvasculature rather than as a direct quantitative measure of endothelial cell number.

Future studies incorporating direct measurements of cerebral blood flow, additional endothelial markers, and aged animal models will be

necessary to more fully define the therapeutic potential of irisin in vascular cognitive impairment. Despite these limitations, the present findings provide preliminary evidence suggesting that irisin may contribute to neurovascular adaptation and cognitive modulation under CCH.

5. Conclusions

In conclusion, the present study demonstrates that CCH is associated with impaired spatial memory and reduced VEGF-positive vascular profiles in the hippocampus. Systemic irisin administration was associated with modest improvements in memory retention and angiogenesis-associated vascular alterations, although its effects on learning parameters were more variable. The observed correlations between VEGF-positive vascular profiles and memory performance support a functional relationship between angiogenic signaling and cognitive outcomes under hypoperfusive conditions.

Beyond its well-established neuroprotective properties, the present findings suggest that irisin may also exert beneficial effects through modulation of angiogenesis-associated vascular signaling in the hippocampus. This neurovascular mechanism may represent an additional pathway contributing to the cognitive effects of irisin under CCH.

Although the magnitude of the observed changes was limited within the four-week treatment period, these results indicate that irisin may contribute to neurovascular adaptation in conditions of CCH. From a translational perspective, irisin may represent a biologically plausible adjunctive strategy for targeting cognitive impairment associated with cerebrovascular pathology, particularly in individuals who are unable to benefit from regular physical exercise. In this respect, the present study provides initial evidence that irisin may function as an exercise-mimetic neurovascular modulator in chronic cerebral hypoperfusion. Further studies are required to clarify the temporal dynamics, endothelial specificity, and molecular mechanisms underlying irisin-mediated neurovascular and cognitive modulation.

CRediT authorship contribution statement

Aslı Karakılıç: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Özgür Bulmuş:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Emrah Özcan:** Writing – review & editing, Visualization, Project administration, Methodology, Formal analysis. **Gülay Turan:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Data curation. **Elif Aksöz:** Writing – review & editing, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Burak Şafak:** Writing – review & editing, Visualization, Supervision, Methodology, Formal analysis, Data curation. **Yunus Emre Özer:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation.

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Declaration of competing interest

The authors have no conflict of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.npep.2026.102613.

Data availability

The data underlying this article are available in https://zenodo.org/records/17376328

References

Candelario-Jalil, E., Paul, S., 2021. Impact of aging and comorbidities on ischemic stroke outcomes in preclinical animal models: a translational perspective. Exp. Neurol. 335, 113494. https://www.ncbi.nlm.nih.gov/pubmed/33035516.

Cao, D., Bai, Y., Li, L., 2018. Common carotid arteries occlusion surgery in adult rats as a model of chronic cerebral hypoperfusion. Bio-Protoc. 8, e2704. https://www.ncbi.nlm.nih.gov/pubmed/34179248.

Cao, L., Ding, W., Feng, Y., Guan, C., Liu, L., Xie, H., Yu, K., Xu, D., Zhao, L., Sha, X., Deng, X., Wu, S., Wang, Y., Wu, Y., Zhang, T., Wang, N., 2025. Effect of electroacupuncture on vascular remodeling in rats with cerebral ischemia by regulating irisin based on VEGF/Akt/eNOS signaling pathway. Brain Res. Bull. 221, 111192. https://www.ncbi.nlm.nih.gov/pubmed/39798935.

Caproni, S., Di Fonzo, A., Colosimo, C., 2025. Oxidative stress: a new pathophysiological pathway in parkinson's disease and a potential target of the brain-sport crosstalk. Parkinsons Dis 2025, 6691390. https://www.ncbi.nlm.nih.gov/pubmed/40162062.

Cechetti, F., Pagnussat, A.S., Worm, P.V., Elsner, V.R., Ben, J., da Costa, M.S., Mestriner, R., Weis, S.N., Netto, C.A., 2012. Chronic brain hypoperfusion causes early glial activation and neuronal death, and subsequent long-term memory impairment. Brain Res. Bull. 87, 109–116. https://www.ncbi.nlm.nih.gov/pubmed/22040859.

Chulakadabba, K., Muangpaisan, W., Siritipakorn, P., Wongviriyawong, T., Suraarunsumrit, P., Chalermsri, C., Intalapaporn, S., Chatthanawaree, W., Srinonprasert, V., Assantachai, P., 2020. Characteristics and real-life outcomes of dementia and cognitive impairment at a geriatric clinic. Dement. Geriatr. Cogn. Disord. 49, 312–320. https://www.ncbi.nlm.nih.gov/pubmed/33075794.

Clark, P.J., Brzezinska, W.J., Puchalski, E.K., Krone, D.A., Rhodes, J.S., 2009. Functional analysis of neurovascular adaptations to exercise in the dentate gyrus of young adult mice associated with cognitive gain. Hippocampus 19, 937–950. https://www.ncbi.nlm.nih.gov/pubmed/19132736.

Damodaran, T., Hassan, Z., Navaratnam, V., Muzaimi, M., Ng, G., Muller, C.P., Liao, P., Dringenberg, H.C., 2014. Time course of motor and cognitive functions after chronic cerebral ischemia in rats. Behav. Brain Res. 275, 252–258. https://www.ncbi.nlm.nih.gov/pubmed/25239606.

Di Carlo, A., Baldereschi, M., Amaducci, L., Lepore, V., Bracco, L., Maggi, S., Bonaiuto, S., Perissinotto, E., Scarlato, G., Farchi, G., Inzitari, D., Group, I.W., 2002. Incidence of dementia, Alzheimer's disease, and vascular dementia in Italy. The ILSA study. J. Am. Geriatr. Soc. 50, 41–48. https://www.ncbi.nlm.nih.gov/pubmed/12028245.

Erdoğan, S., 2024. Determination of sample size using resource equation methods in analysis of variance models in animal studies. Dicle Univ Vet Fak Derg 17, 1–7. https://doi.org/10.47027/duvetfd.1308466.

Ferrara, N., 2004. Vascular endothelial growth factor: basic science and clinical progress. Endocr. Rev. 25, 581–611. https://www.ncbi.nlm.nih.gov/pubmed/15294883.

Fu, P., Chen, Y., Wu, M., Bao, B., Yin, X., Chen, Z., Zhang, M., 2023. Effect of ferroptosis on chronic cerebral hypoperfusion in vascular dementia. Exp. Neurol. 370, 114538. https://www.ncbi.nlm.nih.gov/pubmed/37709116.

Giudice, J., Taylor, J.M., 2017. Muscle as a paracrine and endocrine organ. Curr. Opin. Pharmacol. 34, 49–55. https://www.ncbi.nlm.nih.gov/pubmed/28605657.

Gouveia, K., Hurst, J.L., 2017. Optimising reliability of mouse performance in behavioural testing: the major role of non-aversive handling. Sci. Rep. 7, 44999. https://www.ncbi.nlm.nih.gov/pubmed/28322308.

Guo, M., Yao, J., Li, J., Zhang, J., Wang, D., Zuo, H., Zhang, Y., Xu, B., Zhong, Y., Shen, F., Lu, J., Ding, S., Hu, C., Xu, L., Xiao, J., Ma, X., 2023. Irisin ameliorates age-associated sarcopenia and metabolic dysfunction. J. Cachexia. Sarcopenia Muscle 14, 391–405.

Hai, J., Li, S.T., Lin, Q., Pan, Q.G., Gao, F., Ding, M.X., 2003. Vascular endothelial growth factor expression and angiogenesis induced by chronic cerebral hypoperfusion in rat brain. Neurosurgery 53, 963–970 discussion 970-962. https://www.ncbi.nlm.nih.gov/pubmed/14519228.

Hayashi, T., Noshita, N., Sugawara, T., Chan, P.H., 2003. Temporal profile of angiogenesis and expression of related genes in the brain after ischemia. J. Cereb. Blood Flow Metab. 23, 166–180. https://www.ncbi.nlm.nih.gov/pubmed/12571448.

Iadecola, C., 2013. The pathobiology of vascular dementia. Neuron 80, 844–866. https://www.ncbi.nlm.nih.gov/pubmed/24267647.

Islam, M.R., Valaris, S., Young, M.F., Haley, E.B., Luo, R., Bond, S.F., Mazuera, S., Kitchen, R.R., Caldarone, B.J., Bettio, L.E.B., Christie, B.R., Schmider, A.B., Soberman, R.J., Besnard, A., Jedrychowski, M.P., Kim, H., Tu, H., Kim, E., Choi, S.H., Tanzi, R.E., Spiegelman, B.M., Wrann, C.D., 2021. Exercise hormone irisin is a critical regulator of cognitive function. Nat. Metab. 3, 1058–1070. https://www.ncbi.nlm.nih.gov/pubmed/34417591.

Jodeiri Farshbaf, M., Alvina, K., 2021. Multiple roles in neuroprotection for the exercise derived myokine irisin. Front. Aging Neurosci. 13, 649929. https://www.ncbi.nlm.nih.gov/pubmed/33935687.

Kalaria, R.N., Maestre, G.E., Arizaga, R., Friedland, R.P., Galasko, D., Hall, K., Luchsinger, J.A., Ogunniyi, A., Perry, E.K., Potocnik, F., Prince, M., Stewart, R., Wimo, A., Zhang, Z.X., Antuono, P., World Federation of Neurology Dementia Research, G., 2008. Alzheimer's disease and vascular dementia in developing

countries: prevalence, management, and risk factors. Lancet Neurol. 7, 812–826. https://www.ncbi.nlm.nih.gov/pubmed/18667359.
Kan, T., He, Z., Du, J., Xu, M., Cui, J., Han, X., Tong, D., Li, H., Yan, M., Yu, Z., 2022. Irisin promotes fracture healing by improving osteogenesis and angiogenesis. J Orthop Translat 37, 37–45. https://www.ncbi.nlm.nih.gov/pubmed/36196152.
Kerr, A.L., Steuer, E.L., Pochtarev, V., Swain, R.A., 2010. Angiogenesis but not neurogenesis is critical for normal learning and memory acquisition. Neuroscience 171, 214–226. https://www.ncbi.nlm.nih.gov/pubmed/20804819.
Kuster, O.C., Laptinskaya, D., Fissler, P., Schnack, C., Zugel, M., Nold, V., Thurm, F., Pleiner, S., Karabatsiakis, A., von Einem, B., Weydt, P., Liesener, A., Borta, A., Woll, A., Hengerer, B., Kolassa, I.T., von Arnim, C.A.F., 2017. 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. Alzheimer's Dis 59, 1097–1111. https://www.ncbi.nlm.nih.gov/pubmed/28731451.
Leardini-Tristao, M., Borges, J.P., Freitas, F., Rangel, R., Daliry, A., Tibirica, E., Estato, V., 2017. The impact of early aerobic exercise on brain microvascular alterations induced by cerebral hypoperfusion. Brain Res. 1657, 43–51. https://www.ncbi.nlm.nih.gov/pubmed/27923636.
Lee, E.S., Yoon, J.H., Choi, J., Andika, F.R., Lee, T., Jeong, Y., 2019. A mouse model of subcortical vascular dementia reflecting degeneration of cerebral white matter and microcirculation. J. Cereb. Blood Flow Metab. 39, 44–57. https://www.ncbi.nlm.nih.gov/pubmed/29053032.
Lee, J.M., Park, J., Lee, J.H., Song, M.K., Kim, Y.J., 2021. Preischemic treadmill exercise ameliorates memory impairment and microvasculature damage in rat model of chronic cerebral hypoperfusion. Int. Neurourol. J. 25, S72–S80. https://www.ncbi.nlm.nih.gov/pubmed/34844389.
Leung, W.Y., Jensen, M.B., 2013. Histological quantification of angiogenesis after focal cerebral infarction: a systematic review. ISRN Neurol. 2013, 853737. https://www.ncbi.nlm.nih.gov/pubmed/24490083.
Li, D.J., Li, Y.H., Yuan, H.B., Qu, L.F., Wang, P., 2017. The novel exercise-induced hormone irisin protects against neuronal injury via activation of the akt and ERK1/2 signaling pathways and contributes to the neuroprotection of physical exercise in cerebral ischemia. Metabolism 68, 31–42. https://www.ncbi.nlm.nih.gov/pubmed/28183451.
Liao, Q., Qu, S., Tang, L.X., Li, L.P., He, D.F., Zeng, C.Y., Wang, W.E., 2019. Irisin exerts a therapeutic effect against myocardial infarction via promoting angiogenesis. Acta Pharmacol. Sin. 40, 1314–1321. https://www.ncbi.nlm.nih.gov/pubmed/31061533.
Liu, Y., Liu, Y., Zhang, X., Yan, G., Qi, L., Yong, V.W., Xue, M., 2024. The cerebroprotection and prospects of FNDC5/irisin in stroke. Neuropharmacology 253, 109986. https://www.ncbi.nlm.nih.gov/pubmed/38705569.
MacKay-Lyons, M., Billinger, S.A., Eng, J.J., Dromerick, A., Giacomantonio, N., Hafer-Macko, C., Macko, R., Nguyen, E., Prior, P., Suskin, N., Tang, A., Thornton, M., Unsworth, K., 2020. Aerobic exercise recommendations to optimize best practices in care after stroke: AEROBICS 2019 update. Phys. Ther. 100, 149–156. https://www.ncbi.nlm.nih.gov/pubmed/31596465.
Macrae, I.M., 2011. Preclinical stroke research–advantages and disadvantages of the most common rodent models of focal ischaemia. Br. J. Pharmacol. 164, 1062–1078. https://www.ncbi.nlm.nih.gov/pubmed/21457227.
Morris, R., 1984. Developments of a water-maze procedure for studying spatial learning in the rat. J. Neurosci. Methods 11, 47–60. https://www.ncbi.nlm.nih.gov/pubmed/6471907.
Murakami, H., Takanaga, H., Matsuo, H., Ohtani, H., Sawada, Y., 2000. Comparison of blood-brain barrier permeability in mice and rats using in situ brain perfusion technique. Am. J. Physiol. Heart Circ. Physiol. 279, H1022–H1028. https://www.ncbi.nlm.nih.gov/pubmed/10993764.
Oranger, A., Zerlotin, R., Buccoliero, C., Sanesi, L., Storlino, G., Schipani, E., Kozloff, K. M., Mori, G., Colaianni, G., Colucci, S., Grano, M., 2023. Irisin modulates inflammatory, angiogenic, and osteogenic factors during fracture healing. Int. J. Mol. Sci. 24, 1809. https://www.ncbi.nlm.nih.gov/pubmed/36768133.
Otori, T., Katsumata, T., Muramatsu, H., Kashiwagi, F., Katayama, Y., Terashi, A., 2003. Long-term measurement of cerebral blood flow and metabolism in a rat chronic hypoperfusion model. Clin. Exp. Pharmacol. Physiol. 30, 266–272. https://www.ncbi.nlm.nih.gov/pubmed/12680845.
Pahlavani, H.A., 2023. Exercise therapy to prevent and treat alzheimer's disease. Front. Aging Neurosci. 15, 1243869. https://www.ncbi.nlm.nih.gov/pubmed/37600508.
Palmer, T.D., Willhoite, A.R., Gage, F.H., 2000. Vascular niche for adult hippocampal neurogenesis. J. Comp. Neurol. 425, 479–494. https://www.ncbi.nlm.nih.gov/pubmed/10975875.
Pati, S., Orsi, S.A., Moore, A.N., Dash, P.K., 2009. Intra-hippocampal administration of the VEGF receptor blocker PTK787/ZK222584 impairs long-term memory. Brain Res. 1256, 85–91. https://www.ncbi.nlm.nih.gov/pubmed/19100245.
Prince, M., Bryce, R., Albanese, E., Wimo, A., Ribeiro, W., Ferri, C.P., 2013. The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement. 9, 63–75 e62. https://www.ncbi.nlm.nih.gov/pubmed/23305823.
Qi, C., Song, X., Wang, H., Yan, Y., Liu, B., 2022. The role of exercise-induced myokines in promoting angiogenesis. Front. Physiol. 13, 981577. https://www.ncbi.nlm.nih.gov/pubmed/36091401.
Rajeev, V., Fann, D.Y., Dinh, Q.N., Kim, H.A., De Silva, T.M., Lai, M.K.P., Chen, C.L., Drummond, G.R., Sobey, C.G., Arumugam, T.V., 2022. Pathophysiology of blood brain barrier dysfunction during chronic cerebral hypoperfusion in vascular cognitive impairment. Theranostics 12, 1639–1658. https://www.ncbi.nlm.nih.gov/pubmed/35198062.
Roof, R.L., Hall, E.D., 2000. Gender differences in acute CNS trauma and stroke: neuroprotective effects of estrogen and progesterone. J. Neurotrauma 17, 367–388. https://www.ncbi.nlm.nih.gov/pubmed/10833057.
Roof, R.L., Duvdevani, R., Stein, D.G., 1993. Gender influences outcome of brain injury: progesterone plays a protective role. Brain Res. 607, 333–336. https://www.ncbi.nlm.nih.gov/pubmed/8481809.
Ruan, Q., Huang, Y., Yang, L., Ruan, J., Gu, W., Zhang, X., Zhang, Y., Zhang, W., Yu, Z., 2019. The effects of both age and sex on irisin levels in paired plasma and cerebrospinal fluid in healthy humans. Peptides 113, 41–51. https://www.ncbi.nlm.nih.gov/pubmed/30716355.
Ruitenberg, A., Ott, A., van Swieten, J.C., Hofman, A., Breteler, M.M., 2001. Incidence of dementia: does gender make a difference? Neurobiol. Aging 22, 575–580. https://www.ncbi.nlm.nih.gov/pubmed/11445258.
Schmidt-Kastner, R., 2015. Genomic approach to selective vulnerability of the hippocampus in brain ischemia-hypoxia. Neuroscience 309, 259–279. https://www.ncbi.nlm.nih.gov/pubmed/26383255.
Shahabi, S., Esfarjani, F., Zamani, S., Rarani, F.Z., Rashidi, B., 2024. Evaluating the efficacy of irisin injection in mimicking the molecular responses induced by endurance exercise in mouse liver tissue. Int. J. Prev. Med. 15, 66.
Shao, W.H., Li, C., Chen, L., Qiu, X., Zhang, W., Huang, C.X., Xia, L., Kong, J.M., Tang, Y., 2010. Stereological investigation of age-related changes of the capillaries in white matter. Anat Rec (Hoboken) 293, 1400–1407. https://www.ncbi.nlm.nih.gov/pubmed/20665817.
Simon, N.W., Setlow, B., 2006. Post-training amphetamine administration enhances memory consolidation in appetitive pavlovian conditioning: implications for drug addiction. Neurobiol. Learn. Mem. 86, 305–310. https://www.ncbi.nlm.nih.gov/pubmed/16750404.
Stanojlović, M., Gušević, I., Grković, I., Mitrović, N., Horvat, A., Drakulić, D., 2014. Time-related sex diffrences in cerebral hypoperfusion-induced brain injury. Arch. Biol. Sci. 66, 1673–1680. https://www.scopus.com/inward/record.uri?eid=2-s2.0-84921652306&doi=10.2298%2fABS1404673S&partnerID=40&md5=1bbd25690cb74a969665b04fd60a2604.
Tang, Y., Zhang, Y., Zheng, M., Chen, J., Chen, H., Liu, N., 2018. Effects of treadmill exercise on cerebral angiogenesis and MT1-MMP expression after cerebral ischemia in rats. Brain Behav. 8, e01079. https://www.ncbi.nlm.nih.gov/pubmed/30035384.
Tsai, C.L., Pai, M.C., 2021. Circulating levels of irisin in obese individuals at genetic risk for alzheimer's disease: correlations with amyloid-beta, metabolic, and neurocognitive indices. Behav. Brain Res. 400, 113013. https://www.ncbi.nlm.nih.gov/pubmed/33186636.
Venkat, P., Chopp, M., Chen, J., 2015. Models and mechanisms of vascular dementia. Exp. Neurol. 272, 97–108. https://www.ncbi.nlm.nih.gov/pubmed/25987538.
Wang, J., Yang, C., Wang, H., Li, D., Li, T., Sun, Y., Zhao, M., Ma, J., Hua, W., Yang, Z., 2020. A new rat model of chronic cerebral hypoperfusion resulting in early-stage vascular cognitive impairment. Front. Aging Neurosci. 12, 86. https://www.ncbi.nlm.nih.gov/pubmed/32351379.
Wang, S., Pan, J., 2016. Irisin ameliorates depressive-like behaviors in rats by regulating energy metabolism. Biochem. Biophys. Res. Commun. 474, 22–28. https://www.ncbi.nlm.nih.gov/pubmed/27079240.
Wang, Y., Wu, Z., He, Y., Zeng, X., Gu, Z., Zhou, X., Si, W., Chen, D., 2024. Fat mass and obesity-associated protein regulates RNA methylation associated with spatial cognitive dysfunction after chronic cerebral hypoperfusion. Neuropeptides 105, 102428. https://www.ncbi.nlm.nih.gov/pubmed/38583362.
Wrann, C.D., 2015. FNDC5/irisin - their role in the nervous system and as a mediator for beneficial effects of exercise on the brain. Brain Plast 1, 55–61. https://www.ncbi.nlm.nih.gov/pubmed/28480165.
Wu, Y.F., Jin, K.Y., Wang, D.P., Lin, Q., Sun, J., Su, S.H., Hai, J., 2023. VEGF loaded nanofiber membranes inhibit chronic cerebral hypoperfusion-induced cognitive dysfunction by promoting HIF-1a/VEGF mediated angiogenesis. Nanomedicine 48, 102639. https://www.ncbi.nlm.nih.gov/pubmed/36549557.
Yang, F., Wang, Z., Li, B., He, Y., Du, F., Tian, S., Zhang, Y., Yang, Y., 2021. Irisin enhances angiogenesis of mesenchymal stem cells to promote cardiac function in myocardial infarction via PI3k/Akt activation. Int J Stem Cells 14, 455–464. https://www.ncbi.nlm.nih.gov/pubmed/34456190.
Yang, Y., Torbey, M.T., 2020. Angiogenesis and blood-brain barrier permeability in vascular remodeling after stroke. Curr. Neuropharmacol. 18, 1250–1265.
Yardimci, A., Ertugrul, N.U., Ozgen, A., Ozbeg, G., Ozdede, M.R., Ercan, E.C., Canpolat, S., 2023. Effects of chronic irisin treatment on brain monoamine levels in the hypothalamic and subcortical nuclei of adult male and female rats: an HPLC-ECD study. Neurosci. Lett. 806, 137245.
Zhang, M., Xu, Y., Jiang, L., 2019. Irisin attenuates oxidized low-density lipoprotein impaired angiogenesis through AKT/mTOR/S6K1/Nrf2 pathway. J. Cell. Physiol. 234, 18951–18962. https://www.ncbi.nlm.nih.gov/pubmed/30942905.