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Effects of combined hyperbaric oxygen therapy and moderate- intensity aerobic exercise on working memory in patients with stroke: a randomized controlled trial

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Research Article

Keywords: Stroke, Working memory, Hyperbaric oxygen, Aerobic exercise, Functional near-infrared spectroscopy, Dorsolateral prefrontal cortex

Posted Date: September 27th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10570297/v1

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Additional Declarations: No competing interests reported.

Abstract

Background

Stroke is the second leading cause of disability and death worldwide. Approximately 40%–60% of patients with stroke develop persistent working memory (WM) impairment, which severely compromises daily functioning. Moderate-intensity aerobic exercise (EX) and hyperbaric oxygen (HBO) therapy have been shown to improve poststroke cognition; however, their comparative efficacy and the potential added benefit of combining them remain unclear. We aimed to compare the effects of three intervention regimens on WM in older patients with stroke and explore the underlying mechanisms involving neurotrophic factors and prefrontal oxygenation.

Methods

This randomized controlled trial was conducted at The Sixth Hospital of Wuhan between January and December 2025. Thirty-six older patients with stroke of 6–12 months' duration were randomly assigned to the control (CON), EX, HBO, or combined intervention (HBO + EX) group. All groups underwent a 2-week intervention. Preintervention and postintervention assessments included reaction time (RT) during the n-back task, oxyhemoglobin (O_2Hb) changes in the bilateral dorsolateral prefrontal cortices (DLPFCs) measured using functional near-infrared spectroscopy, and serum brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) levels measured using enzyme-linked immunosorbent assays.

Results

Compared with the CON group, all three intervention groups showed significant reductions in postintervention RT ($p < 0.05$), with significantly greater improvement in the HBO + EX group than in the EX and HBO groups ($p < 0.01$). Serum BDNF and VEGF levels increased significantly in all intervention groups ($p < 0.05$), with the greatest VEGF increase in the HBO + EX group. Mean and peak O_2Hb levels in the left DLPFC (DLPFC-L) decreased significantly in all intervention groups ($p < 0.05$), and these changes independently predicted RT improvement ($p < 0.01$).

Conclusions

Moderate-intensity EX and HBO therapy improved WM in older patients with stroke, and their combination exerted a synergistic effect. These improvements were associated with increased BDNF and VEGF levels and improved oxygenation efficiency in the DLPFC-L. Changes in DLPFC-L oxygenation independently predicted WM recovery.

Trial registration:

ClinicalTrials.gov NCT07772947, registered on August 14, 2026. Retrospectively registered.

1. Background

Stroke is an acute cerebrovascular disease caused by the rupture or occlusion of cerebral vessels, resulting in focal brain tissue damage [1]. It is the second leading cause of disability and death globally [2] and imposes a substantial burden on individuals and society, making exercise-based rehabilitation an active research area [3]. In addition to well-recognized motor deficits, many stroke survivors experience cognitive impairment [4]. Working memory (WM), the capacity to temporarily hold and manipulate information during complex tasks, is frequently affected [5, 6]. WM is essential not only for everyday activities [7] and occupational performance [8] but also for emotional regulation [9] and coping with stress [10]. Importantly, WM dysfunction limits patients' independence in daily living, impedes motor-learning efficiency, reduces the effectiveness of physical rehabilitation, and increases the risk of falls and long-term institutionalization [11]. Therefore, targeted interventions to improve WM are critical for optimizing rehabilitation outcomes and quality of life in patients with stroke.

Aerobic exercise (EX) [12] and hyperbaric oxygen (HBO) therapy [13] have emerged as effective rehabilitation strategies for patients with stroke. Both approaches can increase systemic oxygen delivery, thereby improving neural plasticity [14, 15], cognitive function, and motor recovery in affected limbs. Moderate-intensity EX has been shown to augment frontal neural activity and increase oxygenated hemoglobin (O_2Hb) levels in the frontal cortex through neurovascular coupling [16]. Acute moderate-intensity EX also enhances WM performance [17, 18]. Animal and human studies have demonstrated that exercise increases vascular endothelial growth factor (VEGF) protein levels in skeletal muscle and plasma, thereby promoting angiopoietin receptor expression [19, 20].

HBO therapy, which delivers 100% oxygen at pressures > 1 atm, can increase dissolved oxygen in the blood by 5- to 20-fold [21]. Because oxygen is a rate-limiting factor for brain activity, increased oxygen availability enhances neural activity [22]. Clinical evidence indicates that HBO therapy increases neural stem cell and VEGF levels, thereby promoting neuronal proliferation and angiogenesis [23]. Functional magnetic resonance imaging during WM tasks in healthy young adults after HBO exposure revealed improved WM performance and increased regional homogeneity in the left hippocampus, right inferior frontal gyrus, and lingual gyrus, regions closely associated with cognitive processing [24]. Although both interventions improve WM by augmenting cerebral oxygen perfusion, their underlying mechanisms differ. EX primarily increases cardiac output and cerebral blood flow while inducing the release of myogenic neurotrophic factors from skeletal muscle [25]. In contrast, HBO therapy directly increases cerebral oxygen partial pressure and predominantly stimulates endogenous neurotrophic factor expression in the central nervous system [26]. Given these distinct pathways, we hypothesized that combination therapy would produce synergistic effects superior to those of either monotherapy, thereby ameliorating WM deficits in patients with stroke.

Both EX and HBO therapy can upregulate neurotrophic and angiogenic factors, including brain-derived neurotrophic factor (BDNF) and VEGF. BDNF is critical for neuronal survival, synaptogenesis, and neuroplasticity, and its upregulation in the prefrontal cortex is closely associated with improved WM [27]. EX increases circulating BDNF levels, facilitates synaptic remodeling, and enhances prefrontal cortical function [28]. Similarly, HBO therapy increases BDNF expression in peri-infarct and prefrontal regions, supporting neural repair and cognitive recovery after stroke [29]. VEGF promotes cerebral angiogenesis, improves microvascular perfusion, and enhances neurovascular coupling, all of which are essential for maintaining prefrontal oxygenation and supporting cognitive processing [30]. Exercise and HBO therapy both increase VEGF levels, facilitating new blood vessel formation and improving cerebral blood flow regulation [31]. Collectively, BDNF-mediated neuroplasticity and VEGF-driven angiogenesis may synergistically improve WM.

To noninvasively examine the neural correlates of cognitive improvement, we used functional near-infrared spectroscopy (fNIRS), which monitors cerebral hemodynamics by quantifying changes in O_2Hb [32]. fNIRS can distinguish cognitive loads and provide indirect markers of neural activation [33]. A probe-embedded cap was placed over each participant's head to record cortical activity in the target regions. Because the dorsolateral prefrontal cortex (DLPFC) is a core region supporting WM [34], fNIRS-derived DLPFC oxygenation changes may provide insight into the neural mechanisms underlying intervention-induced WM improvement.

Thus, both interventions increase cerebral oxygen supply—one through enhanced blood flow and the other through elevated dissolved oxygen—and have been shown to improve WM and increase neurotrophic factor levels. However, it remains unclear whether the treatments differ in their effects on WM, BDNF, and VEGF and whether combination therapy produces a synergistic effect beyond either monotherapy. This randomized controlled trial was designed to address these questions and provide evidence for optimizing poststroke cognitive rehabilitation.

2. Methods

2.1. Study design

This randomized controlled trial was conducted at The Sixth Hospital of Wuhan between January and December 2025. Participants were fully informed of the study purpose and safety procedures before enrollment, and written informed consent was obtained from each participant. The study was approved by the Ethics Committee of The Sixth Hospital of Wuhan (approval number: WHSHIRB-K-2024011) and conducted in accordance with the ethical standards of the Declaration of Helsinki. This randomized controlled trial was reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) 2025 guidelines.

2.2. Participants

A total of 36 older patients with stroke were recruited from the Department of Rehabilitation Medicine at The Sixth Hospital of Wuhan. Eligible participants were randomly assigned to one of four groups.

2.2.1. Inclusion criteria

The inclusion criteria [35] were as follows: a first-ever stroke confirmed using computed tomography or magnetic resonance imaging; a stroke duration of 6–12 months; age ≥ 65 years; a Mini-Mental State Examination score ≥ 21; the ability to understand and complete WM tasks; and the ability to tolerate moderate-intensity EX and HBO therapy.

2.2.2. Exclusion criteria

The exclusion criteria [36] were severe aphasia or visual or auditory impairment that precluded task completion; a history of psychiatric disorders; severe cardiopulmonary, hepatic, or renal dysfunction; contraindications to HBO therapy or EX; and participation in another clinical trial within the previous 3 months.

2.3. Experimental grouping

2.3.1. Control group

Participants in the control (CON) group received standard rehabilitation consisting of physical and occupational therapies administered 5 days per week for 2 weeks. The program focused on improving motor function, balance, and activities of daily living and did not include specific cognitive training.

2.3.2. EX group

In addition to standard rehabilitation, participants in the EX group performed moderate-intensity EX on a stationary bicycle (Ergoselect 600; Hanxiang Biology, Shenzhen, China). Exercise intensity was set at 60%–69% of the predicted maximum heart rate, calculated as 220 minus age [3]. Heart rate was continuously monitored using a chest-strap monitor (G3M; Genrui Medical Instruments, Guangdong, China). Resistance was adjusted if the heart rate exceeded the target range. The protocol consisted of a 4-min warm-up, 20 min of moderate-intensity cycling, and a 3-min cool-down, performed 5 days per week for 2 weeks [37, 38].

2.3.3. HBO therapy group

In addition to standard rehabilitation, participants in the HBO group received HBO therapy in a medical air-pressurized oxygen chamber (GY2800D1; Yantai Hongyuan Oxygen Industry, China). Each session involved breathing 100% oxygen through a mask at 2 atmospheres absolute (ATA) for 90 min, with 5-min air breaks every 20 min. The compression and decompression rates were 1 m/min. Treatment was administered 5 days per week for 2 weeks [39, 40].

2.3.4. Combined intervention (HBO + EX) group

Participants in the HBO + EX group received moderate-intensity EX and HBO therapy in addition to standard rehabilitation. To minimize potential sequence effects, participants were randomly assigned to the treatment order. The frequency and duration of each intervention were identical to those in the EX and HBO groups.

2.4. Experimental procedure

The intervention period was 14 days. Participants underwent WM testing and venous blood sampling on day 1 (preintervention) and day 14 (postintervention). Interventions began on day 1 and were delivered five times per week for a total of 10 sessions over 2 weeks.

2.5. WM task

WM was evaluated using the 0-back and 2-back tasks (fNIRS BS-7000; Yiruide, China). Testing was conducted in a quiet, well-lit room with minimal distractions. Participants were seated comfortably approximately 60 cm from a computer screen.

During the task, participants were instructed to minimize head movement, biting, leg shaking, and other movements. The paradigm comprised four 30-s rest blocks and three 32-s task blocks. During rest, participants fixated on a “+” at the center of the screen. During the 2-back task, participants viewed a series of numbers and determined whether each number matched the number presented two positions earlier. They pressed the green button for a match and the red button for a nonmatch (Fig. 1a). During the 0-back task, participants pressed either button immediately after a number appeared (Fig. 1b). Each number was presented for 0.5 s, followed by a “+” for 1.5 s. Each block contained 12 digits, with 10 responses in the 2-back task and 12 responses in the 0-back task. The 2-back and 0-back tasks alternated, with three repetitions of each. Mean reaction time (RT) was the behavioral measure of WM. WM processing time was calculated by subtracting the 0-back RT from the 2-back RT.

2.6. Functional near-infrared spectroscopy

Changes in cerebral oxygenation in the bilateral DLPFCs were measured using a continuous-wave fNIRS system (NIR-800; Yiruide, Wuhan, China) (Fig. 2). The system operated at wavelengths of 730 and 850 nm, with a sampling rate of 10 Hz and an optode separation of 3 cm. The probe array was positioned over the bilateral DLPFCs according to the international 10–20 electrode placement system, using the frontal pole as the anatomical landmark. Stimulus triggers for the n-back task were synchronously logged in the fNIRS data stream to ensure temporal alignment between cognitive events and hemodynamic recordings. Participants remained seated and minimized head movement, swallowing, and blinking during acquisition. A single trained operator performed all procedures.

Raw light-intensity data were exported in comma-separated-value format and preprocessed in MATLAB R2014a using the NirCompass V3.0 analysis script library (Zilian Hongkang Technology Co., Ltd., Wuhan, China) integrated with the Homer2 toolbox. Data were converted to the standard .nirs format, and signal quality was assessed using the baseline coefficient of variation. Channels with a coefficient of variation >

25% were classified as poor-quality channels and excluded from subsequent region-of-interest (ROI) calculations. Signals with saturation artifacts or extreme amplitude jumps were also discarded. Motion artifacts were identified using a sliding-window standard deviation algorithm (0.5-s window, 20-fold standard deviation threshold, and 5-fold amplitude threshold) and corrected using cubic-spline interpolation. A 0.01–0.1-Hz bandpass filter was applied to attenuate low-frequency drift and high-frequency physiological noise from cardiac, respiratory, and muscular sources. Optical density values were converted to relative concentration changes in O_2Hb and deoxygenated hemoglobin using the modified Beer–Lambert law, with a differential pathlength factor of 6.0 for the adult prefrontal cortex. Baseline correction was performed by subtracting the mean signal during the 2-s rest epoch preceding each task block from the task-phase signal to account for interindividual baseline differences.

The bilateral DLPFCs were defined as two ROIs, and O_2Hb signals within each ROI were averaged across all valid channels. Three metrics were extracted from each 32-s 2-back task block: mean concentration (Mean), reflecting overall cerebral activation; peak concentration (Peak), reflecting maximum activation intensity; and slope (K), reflecting the cerebral activation response speed and calculated as the signal rise rate during the 2–7-s window after task onset. The three repeated 2-back trials were averaged for each participant and included in group-level statistical analyses.

2.7. BDNF and VEGF

Fasting venous blood samples (3 mL) were collected from each participant on days 1 and 14 of the intervention. Samples were centrifuged at 3000 rpm for 20 min to separate the serum, which was stored at − 80°C until analysis (DW-86L959BPT; Haier, China).

Serum BDNF and VEGF concentrations were measured using commercially available enzyme-linked immunosorbent assay kits (ELISA9004; Wuhan Sanying Biotechnology, China) according to the manufacturer's instructions.

2.8. Statistical analysis

All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Data are presented as the mean ± standard error. A p value < 0.05 was considered statistically significant.

First, two-way repeated-measures analysis of variance (ANOVA) was used to examine the main effects of group (CON, HBO, EX, and HBO + EX) and time (preintervention vs. postintervention) and their interaction on K, Mean, Peak, RT (2-back minus 0-back), BDNF, and VEGF. When the group × time interaction was significant, one-way ANOVA was used for between-group comparisons at each time point, and paired-samples t tests were used for within-group comparisons.

Second, change scores (postintervention minus preintervention) were calculated for all variables. Separate change scores were calculated for the DLPFC-L O_2Hb indicators. Group differences in change scores were evaluated using one-way ANOVA, followed by Tukey post hoc pairwise comparisons when the main effect was significant.

Third, multiple linear regression analyses were performed to identify independent predictors of WM improvement. Separate models used RT change as the dependent variable and changes in K, mean O_2Hb, peak O_2Hb, BDNF, and VEGF as independent variables.

Finally, Pearson correlation analyses were performed among changes in K, mean O_2Hb, peak O_2Hb, RT, BDNF, and VEGF to clarify the relationships among prefrontal oxygenation, cognitive performance, and neurotrophic factors.

3. Results

3.1. Participant characteristics

A total of 36 patients who met the diagnostic criteria for poststroke cognitive impairment were enrolled. Baseline demographic and clinical characteristics, including age, sex, handedness, side of hemiplegia, and disease duration, are summarized in Table 1. Continuous and categorical variables were compared among groups using one-way ANOVA and the chi-square test, respectively. No baseline characteristic differed significantly among the four groups (*all $p > 0.05$*).

Table 1
Baseline demographic and clinical characteristics of participants

Participants (n)	**CON**	**HBO**	**EX**	**HBO + EX**
	9	**9**	**9**	**9**
Age (y)	75.78 ± 5.74	77.00 ± 5.72	79.33 ± 8.90	78.33 ± 5.02
Sex (M/F)	4/5	6/3	5/4	5/4
Handedness (R)	9	9	9	9
Hemiplegia (L)	9	9	9	9

Note. Data are presented as the mean ± standard deviation for age and as counts for categorical variables. Continuous and categorical variables were compared using one-way analysis of variance and the chi-square test, respectively. No significant between-group differences were observed at baseline *(all $p > 0.05$)*. M, male; F, female; R, right; L, left; CON, control group; EX, aerobic exercise group; HBO, hyperbaric oxygen therapy group; HBO + EX, combined intervention group.

3.2. Intervention effects on WM performance and neurotrophic factors

Preintervention and postintervention RT, BDNF, and VEGF values are presented in Table 2. At baseline, RT, BDNF, and VEGF did not differ significantly among groups (*$p > 0.05$*).

Table 2
Preintervention and postintervention RT, BDNF, and VEGF across the four groups

Indicator	Time point	CON	HBO	EX	HBO + EX
RT (ms)	pre	991.007 ± 53.691	985.147 ± 81.773	1071.149 ± 67.251	1028.944 ± 51.669
	post	983.192 ± 29.375	**738.789 ± 38.158***†	**660.643 ± 35.816***†	**533.863 ± 20.738***†‡§
BDNF (pg/mL)	pre	2803.462 ± 307.580	2711.468 ± 262.038	2771.318 ± 241.377	2796.466 ± 402.633
	post	2602.413 ± 393.454	**13467.965 ± 1439.242***†	**16542.099 ± 1784.866***†	**14318.019 ± 981.906***†
VEGF (pg/mL)	pre	204.463 ± 10.000	214.795 ± 14.722	222.424 ± 19.586	212.407 ± 20.391
	post	200.126 ± 9.752	**442.380 ± 16.305***†§	**307.045 ± 18.736***†	**625.923 ± 59.092***†‡§

Note. Data are presented as the mean ± standard error. RT, reaction time; BDNF, brain-derived neurotrophic factor; VEGF, vascular endothelial growth factor; CON, control group; EX, aerobic exercise group; HBO, hyperbaric oxygen therapy group; HBO + EX, combined intervention group. *p < 0.05 vs. preintervention within the same group; †p < 0.01 vs. preintervention within the same group; ‡p < 0.05 vs. the HBO group at postintervention; §p < 0.05 vs. the CON group at postintervention.

RT did not change significantly in the CON group. RT decreased significantly from preintervention to postintervention in all three intervention groups ($p < 0.05$). Post hoc comparisons showed that postintervention RT was significantly lower in the HBO, EX, and HBO + EX groups than in the CON group ($p < 0.05$) and significantly lower in the HBO + EX group than in the HBO and EX groups ($p < 0.05$).

BDNF and VEGF levels did not change significantly in the CON group. Both levels increased significantly after intervention in all three intervention groups ($p < 0.05$) and were significantly higher than those in the CON group ($p < 0.05$). Postintervention VEGF levels were significantly higher in the HBO + EX group than in the HBO and EX groups ($p < 0.05$).

[Insert Table 2]

3.3. Intervention effects on prefrontal cortical oxygenation

Preintervention and postintervention O_2Hb indicators in the bilateral DLPFCs are shown in Table 3. At baseline, O_2Hb K, mean, and peak values did not differ significantly among groups in either the DLPFC-L or the right DLPFC (DLPFC-R) ($p > 0.05$).

Table 3
Preintervention and postintervention oxyhemoglobin indicators in the bilateral dorsolateral prefrontal cortices

Brain region	Indicator	Time point	CON	HBO	EX	HBO + EX
DLPFC-L	K	pre	−0.001 ± 0.001	−0.001 ± 0.002	−0.001 ± 0.001	−0.002 ± 0.002
	(a.u.)	post	0.000 ± 0.002	−0.001 ± 0.002	0.000 ± 0.001	**−0.001 ± 0.001***
	Mean	pre	0.007 ± 0.027	0.052 ± 0.022	0.041 ± 0.025	0.032 ± 0.022
	(mM·mm)	post	0.008 ± 0.027	**−0.003 ± 0.018*†**	**−0.022 ± 0.024*†**	**−0.025 ± 0.016*†**
	Peak	pre	0.015 ± 0.044	0.113 ± 0.043	0.090 ± 0.039	0.038 ± 0.059
	(mM·mm)	post	0.010 ± 0.047	**−0.019 ± 0.028***	**−0.021 ± 0.045***	**−0.011 ± 0.036***
DLPFC-R	K	pre	0.000 ± 0.001	−0.002 ± 0.001	−0.001 ± 0.001	−0.002 ± 0.002
	(a.u.)	post	0.000 ± 0.002	−0.002 ± 0.001	−0.001 ± 0.002	−0.002 ± 0.001
	Mean	pre	0.020 ± 0.018	−0.002 ± 0.031	0.045 ± 0.020	0.025 ± 0.020
	(mM·mm)	post	0.019 ± 0.019	0.014 ± 0.014	0.002 ± 0.018	**0.008 ± 0.020***
	Peak	pre	0.032 ± 0.030	0.002 ± 0.002	0.075 ± 0.038	0.028 ± 0.049
	(mM·mm)	post	0.017 ± 0.040	**0.022 ± 0.032***	−0.001 ± 0.041	**0.016 ± 0.038***

Note. Data are presented as the mean ± standard error. K, slope of O_2Hb signal rise; Mean, mean O_2Hb concentration; Peak, peak O_2Hb concentration; DLPFC-L, left dorsolateral prefrontal cortex; DLPFC-R, right dorsolateral prefrontal cortex; CON, control group; EX, aerobic exercise group; HBO, hyperbaric oxygen therapy group; HBO + EX, combined intervention group. *$p < 0.05$ vs. preintervention within the same group.

In the DLPFC-L, no O_2Hb indicator changed significantly in the CON group. Postintervention mean and peak O_2Hb values were significantly lower than preintervention values in the HBO, EX, and HBO + EX groups ($p < 0.05$) and significantly lower than those in the CON group ($p < 0.05$). Only the HBO + EX group showed a significant postintervention reduction in K ($p < 0.05$).

In the DLPFC-R, no O_2Hb indicator changed significantly in the CON or EX group. The HBO group showed a significant postintervention increase in the peak value ($p < 0.05$), whereas the HBO + EX group showed significant increases in the mean and peak values ($p < 0.05$). No postintervention value differed significantly among groups.

[Insert Table 3]

3.4. Changes in RT and neurotrophic factors

RT change differed significantly among groups (one-way ANOVA: F = 12.106, $p < 0.01$). RT reductions were significantly greater in the HBO, EX, and HBO + EX groups than in the CON group ($p < 0.05$) and significantly greater in the HBO + EX group than in the HBO and EX groups ($p < 0.01$) (Fig. 3a).

BDNF change differed significantly among groups (one-way ANOVA: F = 20.070, $p < 0.01$). BDNF increases were significantly greater in all three intervention groups than in the CON group ($p < 0.01$), with no significant difference among the intervention groups (Fig. 3b).

VEGF change differed significantly among groups (one-way ANOVA: F = 29.544, $p < 0.01$). VEGF increases were significantly greater in the HBO and HBO + EX groups than in the CON and EX groups ($p < 0.05$) (Fig. 3c).

3.5. Changes in O_2Hb

K change did not differ significantly among groups in either the DLPFC-L (F = 0.138, $p = 0.937$) or the DLPFC-R (F = 0.138, $p = 0.937$) (Fig. 4a).

Mean O_2Hb change differed significantly among groups in the DLPFC-L (F = 1.289, $p < 0.05$). Mean reductions were significantly greater in the HBO, EX, and HBO + EX groups than in the CON group ($p < 0.05$). Mean O_2Hb change did not differ significantly among groups in the DLPFC-R (F = 1.104, $p = 0.362$) (Fig. 4b).

Peak O_2Hb change differed significantly among groups in the DLPFC-L (F = 1.308, $p < 0.05$). Peak reductions were significantly greater in the HBO and EX groups than in the CON group ($p < 0.05$). Peak O_2Hb change did not differ significantly among groups in the DLPFC-R (F = 0.930, $p = 0.438$) (Fig. 4c).

3.6. Multiple linear regression analysis

In the DLPFC-L models, mean change (β = 0.262, $p < 0.01$) and peak change (β = 0.414, $p < 0.01$) independently predicted RT change, indicating that DLPFC-L oxygenation changes predicted the degree of WM improvement (Table 4). The positive standardized regression coefficients indicated that a smaller reduction in O_2Hb concentration (i.e., a larger change value) was associated with a smaller reduction in RT (i.e., less WM improvement). This dose–response relationship supports the neural-efficiency hypothesis: greater improvement in prefrontal neural efficiency, manifested as a larger decrease in task-related O_2Hb, corresponded to greater cognitive improvement. BDNF and VEGF changes did not

significantly predict RT change in any model ($p > 0.05$), and no DLPFC-R O_2Hb change significantly predicted RT change ($p > 0.05$).

Table 4
Multiple linear regression analysis of predictors for working memory reaction time changes

Brain region	Predictor variable	Unstandardized B	SE	Standardized β	t-value	*p*-value	95% CI (lower–upper)
DLPFC-L							
O_2Hb K model	BDNF change	−0.007	0.009	−0.174	−0.738	0.466	−0.025 to 0.012
	VEGF change	0.21	0.269	0.153	0.781	0.441	−0.342 to 0.762
	O_2Hb K change	5771.591	5672.13	0.147	1.018	0.318	−5847.240 to 17390.422
O_2Hb Mean model	BDNF change	−0.009	0.006	−0.243	−1.577	0.126	−0.022 to 0.003
	VEGF change	0.242	0.168	0.176	1.441	0.161	−0.102 to 0.586
	O_2Hb mean change	**837.346**	**214.359**	**0.262**	**3.906**	**< 0.01**	**398.251 to 1276.441**
O_2Hb Peak model	BDNF change	−0.004	0.005	−0.108	−0.818	0.420	−0.015 to 0.006
	VEGF change	−0.067	0.157	−0.049	−0.428	0.672	−0.389 to 0.255
	O_2Hb peak change	**644.777**	**110.063**	**0.414**	**5.858**	**< 0.01**	**419.324 to 870.230**
DLPFC-R							
O_2Hb K model	BDNF change	−0.011	0.007	−0.288	−1.552	0.132	−0.026 to 0.004
	VEGF change	0.261	0.219	0.19	1.195	0.242	−0.187 to 0.710
	O_2Hb K change	8528.36	6165.335	0.12	1.383	0.178	−4100.756 to 21157.476

Note. Dependent variable: RT change (postintervention minus preintervention). Independent variables: changes in O_2Hb indicators (K, mean, and peak) in the bilateral DLPFCs, BDNF change, and VEGF change. DLPFC-L, left dorsolateral prefrontal cortex; DLPFC-R, right dorsolateral prefrontal cortex; O_2Hb-K, O_2Hb-mean, and O_2Hb-peak, changes in the slope, mean, and peak oxyhemoglobin values, respectively; B, unstandardized regression coefficient; SE, standard error; β, standardized regression coefficient; CI, confidence interval; RT, reaction time. $p < 0.01$ indicates a statistically significant predictive effect.

Brain region	Predictor variable	Unstandardized B	SE	Standardized β	t-value	*p*-value	95% CI (lower–upper)
O_2Hb Mean model	BDNF change	−0.009	0.007	−0.229	−1.181	0.248	−0.024 to 0.006
	VEGF change	0.176	0.248	0.128	0.708	0.485	−0.332 to 0.684
	O_2Hb mean change	631.148	437.785	0.177	1.442	0.160	−265.613 to 1527.910
O_2Hb Peak model	BDNF change	−0.008	0.008	−0.215	−1.051	0.302	−0.024 to 0.008
	VEGF change	0.163	0.273	0.118	0.596	0.556	−0.397 to 0.722
	O_2Hb peak change	380.365	308.924	0.183	1.231	0.228	−252.437 to 1013.167

Note. Dependent variable: RT change (postintervention minus preintervention). Independent variables: changes in O_2Hb indicators (K, mean, and peak) in the bilateral DLPFCs, BDNF change, and VEGF change. DLPFC-L, left dorsolateral prefrontal cortex; DLPFC-R, right dorsolateral prefrontal cortex; O_2Hb-K, O_2Hb-mean, and O_2Hb-peak, changes in the slope, mean, and peak oxyhemoglobin values, respectively; B, unstandardized regression coefficient; SE, standard error; β, standardized regression coefficient; CI, confidence interval; RT, reaction time. $p < 0.01$ indicates a statistically significant predictive effect.

[Insert Table 4]

3.7. Pearson correlation analysis

All significant correlations were observed in the DLPFC-L in the three intervention groups. No indicator was significantly correlated in the CON group or in the DLPFC-R ($p > 0.05$). Detailed results are presented in Table 5.

In the HBO group, mean change (r = − 0.766, $p < 0.05$) and peak change (r = − 0.668, $p < 0.05$) were negatively correlated with RT change, indicating that a greater reduction in DLPFC-L oxygenation was associated with greater RT improvement. In the EX group, mean change (r = 0.711, $p < 0.05$) and peak change (r = 0.717, $p < 0.05$) were positively correlated with RT change. In the HBO + EX group, K change (r = 0.688, $p < 0.05$) and peak change (r = 0.686, $p < 0.05$) were positively correlated with RT change.

In the HBO group, K change (r = 0.685, $p < 0.05$) and peak change (r = 0.765, $p < 0.05$) were positively correlated with VEGF change, whereas peak change was negatively correlated with BDNF change (r = − 0.710, $p < 0.05$). No DLPFC-L O_2Hb indicator was significantly correlated with BDNF change in the EX or HBO + EX group ($p > 0.05$).

Table 5
Pearson correlation coefficients between DLPFC-L O_2Hb changes and changes in RT, BDNF, and VEGF

O_2Hb vs. RT	**HBO**	**EX**	**HBO + EX**
K change	−0.076	−0.241	**0.688***
Mean change	**−0.766***	**0.711***	0.546
Peak change	**−0.668***	**0.717***	**0.686***
O_2Hb vs. BDNF			
K change	−0.347	−0.132	−0.235
Mean change	−0.522	−0.311	−0.256
Peak change	**−0.710***	−0.452	−0.383
O_2Hb vs. VEGF			
K change	**0.685***	−0.132	−0.066
Mean change	0.450	−0.608	0.430
Peak change	**0.765***	−0.463	0.007

Note. Values are Pearson correlation coefficients (r). DLPFC-L, left dorsolateral prefrontal cortex; RT, reaction time; BDNF, brain-derived neurotrophic factor; VEGF, vascular endothelial growth factor; O_2Hb, oxyhemoglobin; EX, aerobic exercise group; HBO, hyperbaric oxygen therapy group; HBO + EX, combined intervention group. *$p < 0.05$.

4. Discussion

We investigated the effects of moderate-intensity EX, HBO therapy, and their combination on WM in older patients with stroke. The principal findings were as follows: (1) all three interventions significantly shortened RT, with greater improvement after combined treatment than after either monotherapy; (2) BDNF and VEGF levels increased significantly in all intervention groups, and VEGF changes were significantly greater in the HBO and HBO + EX groups than in the EX group; (3) mean and peak O_2Hb levels in the DLPFC-L decreased significantly, whereas no significant overall change was observed in the DLPFC-R; and (4) DLPFC-L oxygenation changes independently predicted WM improvement and were correlated with changes in neurotrophic factors.

4.1. Effects on WM

RT decreased significantly after intervention in all three treatment groups, and postintervention RT was significantly lower in the HBO + EX group than in the HBO and EX groups. Thus, combination therapy

improved WM more effectively than either intervention alone, supporting a synergistic effect of HBO therapy and EX. The RT change analysis further supported this conclusion. These findings are consistent with previous evidence that EX and HBO therapy promote neuroplasticity through distinct pathways [41] and may provide additive or synergistic benefits when combined.

This synergistic effect may arise from the complementary actions of the two interventions. HBO therapy ameliorates cerebral hypoxia by increasing dissolved oxygen in the blood and thereby providing oxygen for neural repair [42]. EX increases cerebral blood flow, promotes BDNF release, and enhances neuroplasticity [43]. In combination, these interventions may optimize oxygen delivery and amplify neural repair, thereby improving WM.

4.2. Differential expression of BDNF and VEGF

All three intervention groups showed significant postintervention increases in BDNF and VEGF. Although the EX group had higher postintervention BDNF levels and larger BDNF changes than the other groups, these differences were not statistically significant, possibly because the small sample was insufficient to detect modest between-group differences. The HBO + EX group also did not have higher BDNF levels than the other intervention groups, suggesting that the complementary effects of combination therapy may not be reflected prominently in circulating BDNF. EX primarily induces the release of muscle-derived BDNF into the circulation through skeletal muscle contraction [44], whereas HBO promotes BDNF expression through neural stem cells and glial cells in the central nervous system [45]. Because these pathways may not be simply additive, their combined effects on systemic BDNF levels may be limited.

Although VEGF increased significantly after intervention in the EX group, the change did not differ significantly from that in the CON group. This finding may reflect the limited capacity of exercise to stimulate systemic VEGF expression because its effects are mainly localized to muscle angiogenesis and postexercise tissue repair [46], with weak and transient increases in circulating VEGF [47]. In contrast, the HBO + EX group had significantly higher postintervention VEGF levels than the HBO and EX groups. The complementary effect may involve HBO-induced upregulation of VEGF transcription through the hypoxia-inducible factor 1-alpha pathway [48] and EX-induced enhancement of VEGF secretion from vascular endothelial cells through increased shear stress [49]. Sustained activation of the hypoxia-inducible factor 1-alpha pathway combined with mechanical shear stress may synergistically promote VEGF synthesis and release.

4.3. Dorsolateral prefrontal cortex

The main oxygenation finding was a significant postintervention reduction in mean and peak O_2Hb in the DLPFC-L, whereas the DLPFC-R showed heterogeneous activation patterns, including significant increases in the HBO and HBO + EX groups. This lateralized effect is consistent with evidence identifying the DLPFC-L as a key associative region supporting WM [50], whereas the DLPFC-R may contribute to compensatory activation after unilateral brain injury.

The reductions in mean and peak O_2Hb may reflect improved neural efficiency. Enhanced neural plasticity and network efficiency after intervention may reduce the neural recruitment required to perform the same task, resulting in lower O_2Hb levels [51]. This interpretation is supported by the multiple linear regression analysis, in which DLPFC-L O_2Hb changes independently predicted WM improvement.

Notably, only mean O_2Hb in the DLPFC-L showed a significant postintervention reduction relative to the CON group. Older patients with stroke may have reduced neuronal reserve, and baseline peak O_2Hb levels may therefore already be low, leaving limited capacity for further reduction [52].

Mean change did not differ significantly among the intervention groups, and peak change did not differ significantly between the HBO + EX and CON groups. Thus, combination therapy did not show a clear complementary effect on DLPFC-L oxygenation. Nevertheless, the HBO + EX group showed a decreasing trend in peak change and a significantly greater RT improvement than the HBO and EX groups. The superior effect of combination therapy may therefore reflect broader improvements in neural efficiency rather than local O_2Hb reduction alone [53]. A single intervention may enhance local neural activity by increasing cerebral blood flow and oxygen supply, whereas combination therapy may activate both BDNF-mediated synaptic remodeling and VEGF-mediated angiogenesis [54]. This dual activation may improve neuronal function, functional connectivity, and global network organization [55], effects that may not be fully captured by local O_2Hb changes but may enhance cognitive performance [56].

4.4. Correlations between neurotrophic factors, O_2Hb, and WM

Correlations between RT and O_2Hb changes were concentrated in the mean and peak O_2Hb measures. The direction differed among intervention groups: mean and peak changes were negatively correlated with RT change in the HBO group but positively correlated in the EX and HBO + EX groups. This opposing pattern may reflect differential effects of HBO therapy and EX on neurovascular coupling [57].

HBO therapy may correct chronic cerebral hypoxia by increasing arterial oxygen partial pressure to > 10 times the normal level at 2 ATA [58]. This supraphysiological oxygen concentration induces cerebral vasoconstriction and reduces basal cerebral blood flow [59]. During the n-back task, increased neural activity requires greater oxygen delivery; however, constricted vessels may not dilate sufficiently to meet metabolic demands, producing neurovascular coupling imbalance in some patients. This mechanism may explain the negative correlation between excessive task-related oxygenation reduction and poor cognitive improvement [60].

In contrast, EX improves cerebral perfusion by increasing cardiac output and promoting vasodilation while enhancing neurovascular coupling to better match oxygen delivery to neural activity [61]. Consequently, reductions in mean and peak changes may reflect increased neural efficiency and improved cognitive function [62]. This distinction may also explain why the HBO + EX group showed greater RT improvement than the monotherapy groups: combination therapy may preserve the oxygen-delivery benefits of HBO while incorporating the neural-efficiency advantages of EX [63].

A significant negative correlation between peak O_2Hb change and BDNF change was observed only in the HBO group, indicating that smaller task-related peak O_2Hb reductions were associated with greater BDNF increases. HBO may already provide sufficient oxygen to brain tissue, such that BDNF expression is directed less toward neuronal survival and more toward cerebrovascular regulation and cerebral blood flow homeostasis [64]. BDNF modulates nitric oxide synthase activity through TrkB receptor activation in endothelial cells, thereby maintaining vascular tone and autoregulatory capacity [65]. Higher BDNF levels may be associated with stronger cerebrovascular autoregulation and smaller task-related cerebral blood flow fluctuations, resulting in smaller peak changes [66].

Peak O_2Hb change was also positively correlated with VEGF change in the HBO group, indicating that greater peak reductions were associated with larger VEGF increases. This finding is consistent with the role of VEGF in promoting collateral circulation and neovascularization and thereby increasing basal cerebral oxygen reserves [67]. During the n-back task, patients with sufficient basal oxygen supply may require less additional blood flow to meet metabolic demands, resulting in smaller peak changes [68].

The correlation pattern between RT and O_2Hb changes in the HBO + EX group resembled that in the EX group, although the correlations were consistently weaker. HBO therapy may affect the VEGF pathway more strongly than EX alone [69]. HBO-induced VEGF upregulation promotes collateral circulation and alters baseline cerebral blood flow [70], which may attenuate the exercise-induced neurovascular response and weaken the relationship between O_2Hb and RT changes [71]. Combination therapy also activates BDNF-mediated synaptic remodeling and VEGF-driven angiogenesis; their synergistic effects may be more evident in network-level functional integration than in linear relationships between local oxygenation and behavioral performance [72]. This may explain the absence of significant correlations between neurotrophic factors and O_2Hb changes in the combined group [73].

4.5. Integrative summary and mechanistic model

In summary, HBO therapy primarily increases oxygen delivery and promotes angiogenesis, although its potential to disrupt neurovascular coupling may limit further efficacy. EX improves neural efficiency through physiological regulation of neurovascular coupling but has more limited effects on systemic oxygen delivery [74]. Combination therapy may produce a synergistic effect by rapidly improving cerebral oxygenation while preserving and enhancing exercise-induced neurovascular regulation [75]. These findings support combined HBO and EX as a potential strategy for poststroke WM rehabilitation and provide mechanistic insight into their complementary actions.

4.6. Limitations

This study has several limitations. First, the sample was small (n = 36), which may have limited the power to detect modest between-group differences and precluded subgroup analyses by stroke characteristics, such as lesion location or severity. Second, the 2-week intervention period precluded evaluation of the long-term efficacy and durability of the observed improvements. Studies with larger samples and longer follow-up are needed to confirm the durability of the apparent synergistic effects. Third, only WM was assessed; effects on other cognitive domains, including executive function, attention, and processing

speed, remain unknown. Fourth, serum BDNF and VEGF were used as proxy markers of neuroplasticity and angiogenesis, and no invasive or imaging-based assessment of cerebral structural or vascular changes was performed, limiting mechanistic inference. Finally, all participants were recruited from a single center, which may limit generalizability. Multicenter studies involving diverse populations are needed to validate and extend these findings.

5. Conclusions

Moderate-intensity EX, HBO therapy, and their combination significantly improved WM in older patients with stroke, with a greater effect after combined treatment than after either monotherapy. All three interventions increased serum BDNF and VEGF levels; EX had a strong effect on BDNF, whereas combined therapy produced the greatest VEGF increase. The interventions improved DLPFC-L oxygenation, and these changes independently predicted WM improvement. The intervention modalities produced distinct neurovascular coupling patterns, and combined therapy may improve cognitive rehabilitation by integrating the oxygen-delivery benefits of HBO with the physiological neural-regulatory effects of EX. These findings support combined HBO and EX as a potential strategy for poststroke cognitive rehabilitation. Larger studies with longer follow-up and broader cognitive assessments are needed to validate these findings and optimize intervention protocols.

Abbreviations

WM, working memory

EX, aerobic exercise

HBO, hyperbaric oxygen

CON, control

RT, reaction time

O_2Hb, oxyhemoglobin

DLPFC, dorsolateral prefrontal cortex

DLPFC-L, left dorsolateral prefrontal cortex

DLPFC-R, right dorsolateral prefrontal cortex

BDNF, brain-derived neurotrophic factor

VEGF, vascular endothelial growth factor

ANOVA, analysis of variance

ROI, region of interest

fNIRS, functional near-infrared spectroscopy

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of The Sixth Hospital of Wuhan (approval number: WHSHIRB-K-2024011). All participants were informed of the study purpose, procedures, and potential risks before enrollment, and written informed consent was obtained from each participant. All procedures were conducted in accordance with the ethical standards of the Declaration of Helsinki.

Consent for publication

Not applicable. The study contains no identifiable personal information or clinical images of individual participants.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by the Wuhan Medical Science Research Project of the Wuhan Municipal Health Commission (grant number: WX23Q45). The funder had no role in study design, data collection or analysis, the decision to publish, or manuscript preparation.

Author Contribution

WQ and YH contributed equally as co-first authors. WQ, YH, and LH: conceptualization, study design, and critical revision of the manuscript. LG: critical review and revision of the manuscript. ZG, LY, and SC: participant recruitment, intervention implementation, and clinical data collection. JY, GR, and GY: functional near-infrared spectroscopy data acquisition and signal preprocessing. NW and YL: serum sample processing and biochemical index detection. ZL and CZ: statistical analysis and interpretation of results. WQ and YH: drafting of the original manuscript. LH: project supervision and final approval of the manuscript. All authors read and approved the final manuscript.

Acknowledgement

The authors thank all participants and the medical staff of the Department of Rehabilitation Medicine at The Sixth Hospital of Wuhan for supporting implementation of the intervention. The authors also thank

the technical team for assistance with functional near-infrared spectroscopy data processing.

Data Availability

The datasets generated and analyzed during the current study are not publicly available due to the inclusion of identifiable clinical data of participants and institutional data protection regulations. De-identified datasets are available from the corresponding author upon reasonable request.

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Figures

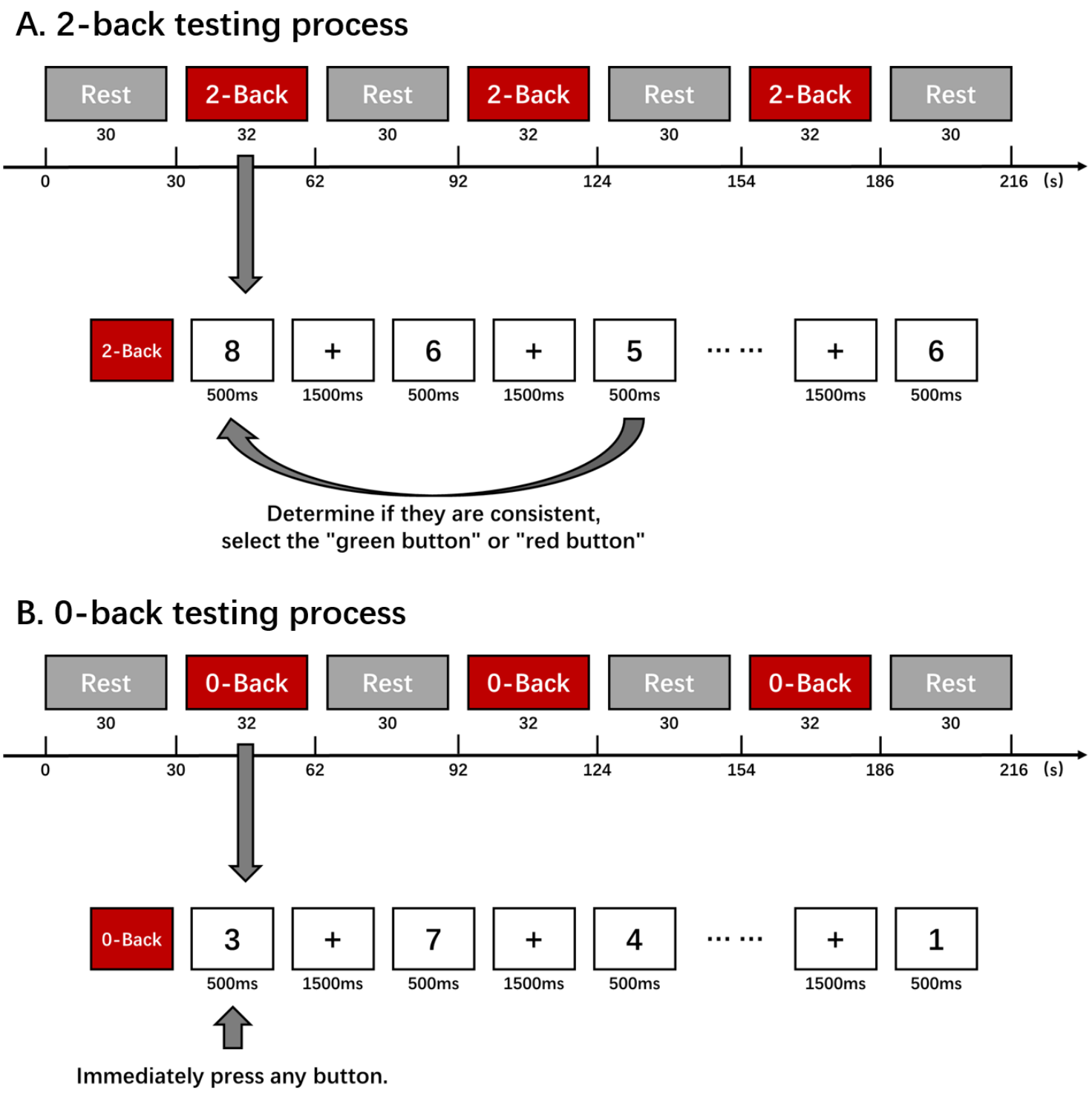


Figure 1

Schematic illustration of the n-back task paradigm

(a) The 2-back task comprised alternating 30-s rest blocks and 32-s task blocks. Each digit was presented for 500 ms, followed by a fixation cross for 1500 ms. Participants pressed the corresponding button to indicate whether the current digit matched the digit presented two trials earlier. (b) During the 0-back task, participants pressed either button immediately when each digit appeared.

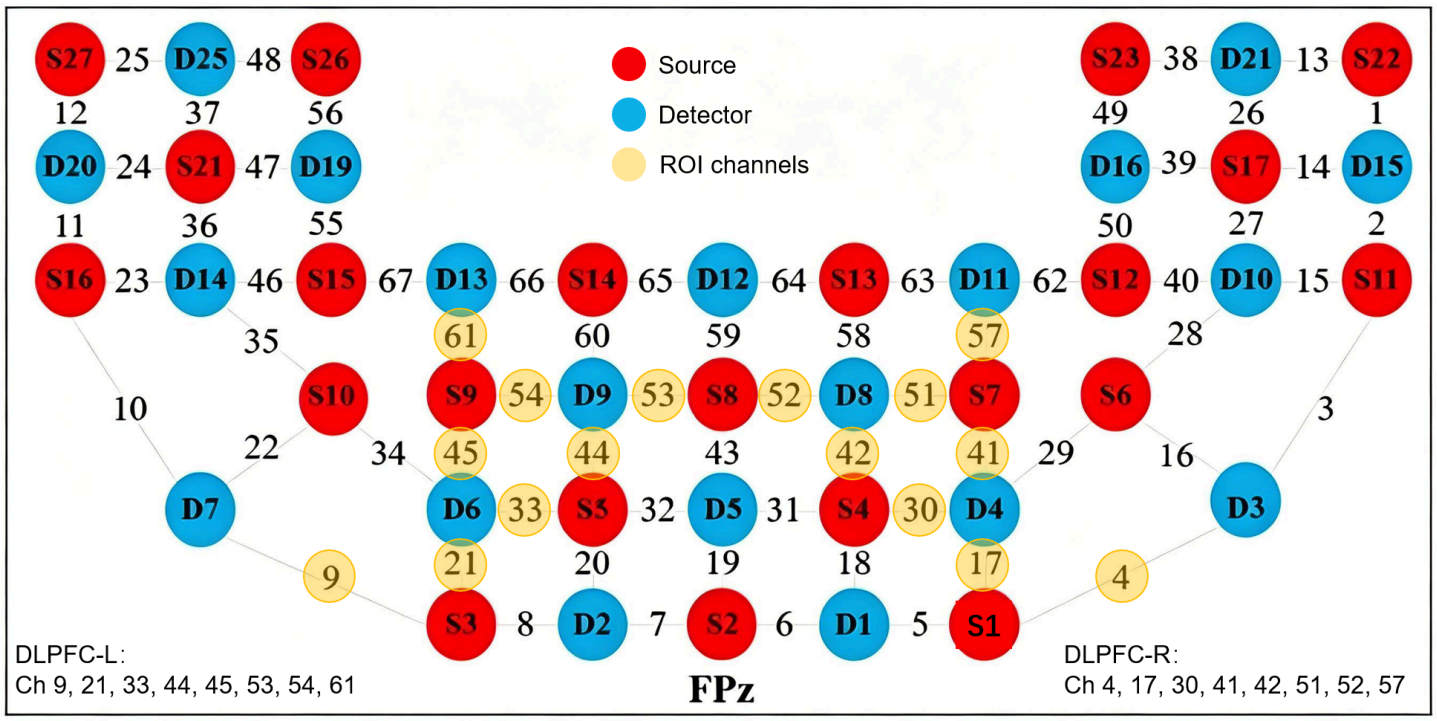


Figure 2

Functional near-infrared spectroscopy optode array and regions of interest

Red circles represent light sources, blue circles represent detectors, and yellow channels indicate the regions of interest (ROIs) covering the bilateral DLPFCs. The image shows a superior view of the cerebral cortex, with FPz oriented downward. The DLPFC-L ROI includes channels 4, 17, 30, 41, 42, 51, 52, and 57; the DLPFC-R ROI includes channels 9, 21, 33, 44, 45, 53, 54, and 61.

ROI, region of interest; DLPFC, dorsolateral prefrontal cortex; DLPFC-L, left dorsolateral prefrontal cortex; DLPFC-R, right dorsolateral prefrontal cortex; FPz, frontal pole.

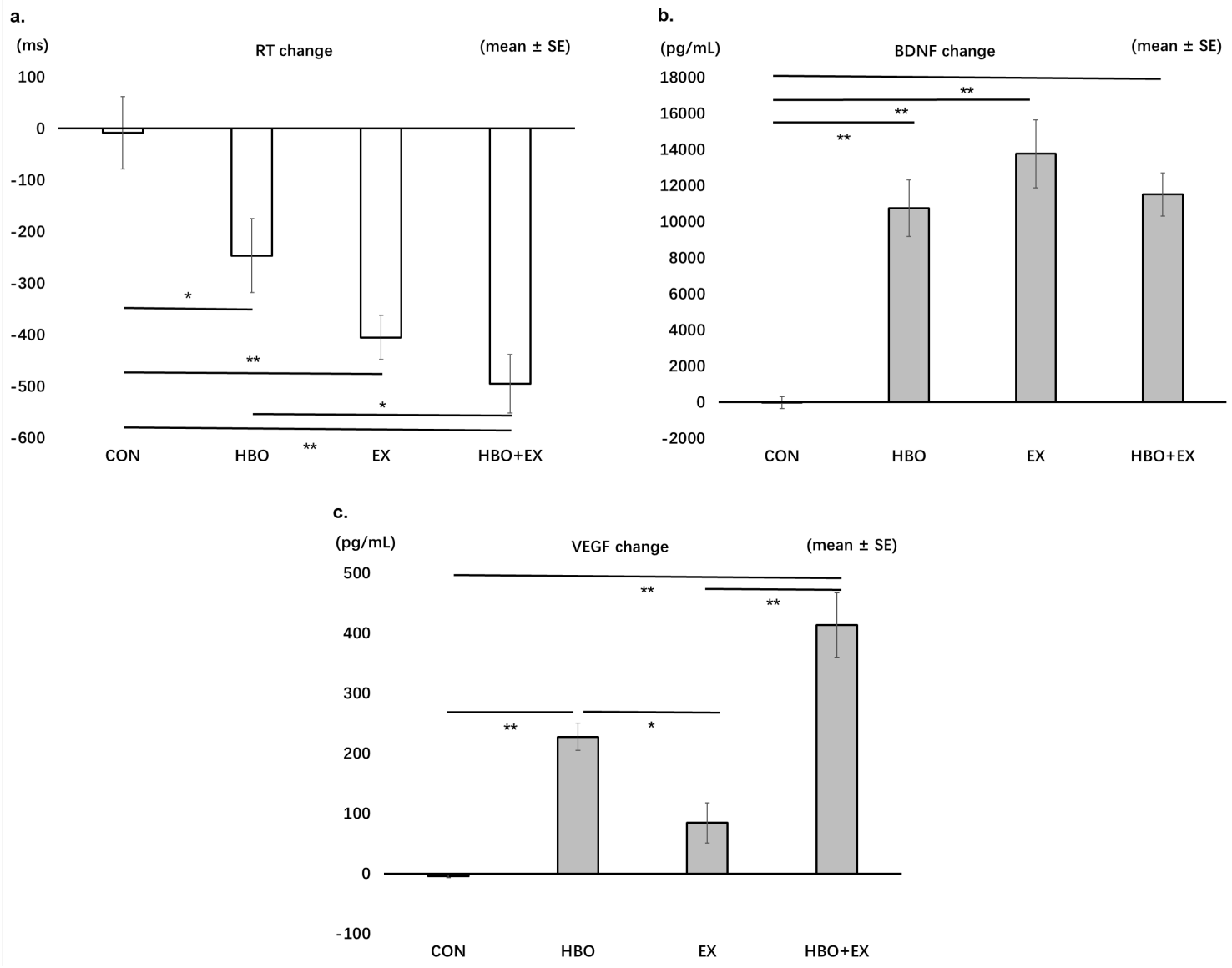


Figure 3

Changes in working memory performance and neurotrophic factor levels

(a) Change in RT; (b) change in BDNF concentration; and (c) change in VEGF concentration. Data are presented as the mean ± standard error. Negative values indicate reduced RT (improved working memory), whereas positive values indicate increased neurotrophic factor levels. *$p < 0.05$; **$p < 0.01$.

RT, reaction time; BDNF, brain-derived neurotrophic factor; VEGF, vascular endothelial growth factor; CON, control group; EX, aerobic exercise group; HBO, hyperbaric oxygen therapy group; HBO+EX, combined intervention group.

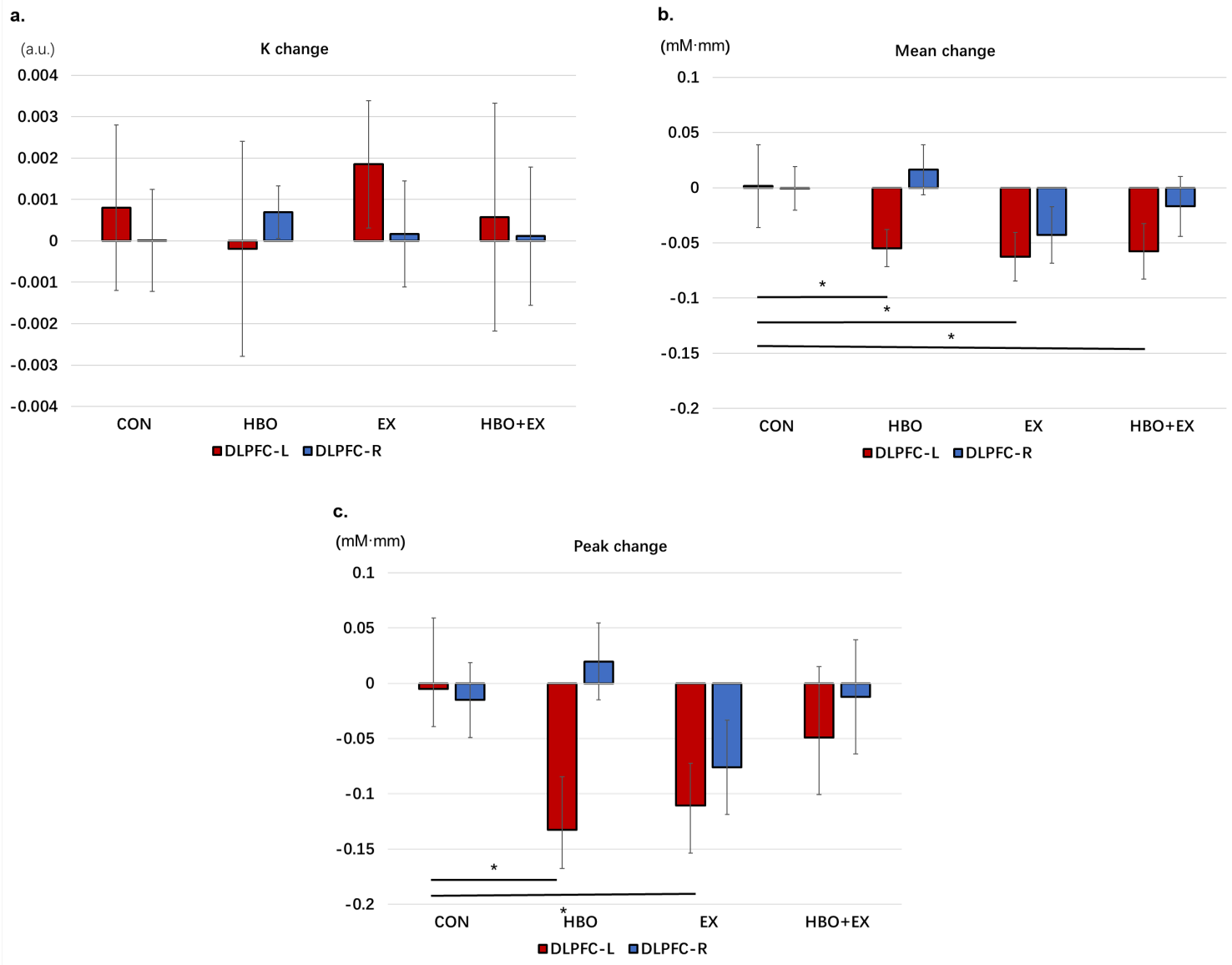


Figure 4

Oxyhemoglobin changes in the bilateral dorsolateral prefrontal cortices

(a) Change in signal slope (K); (b) change in mean oxyhemoglobin (O_2Hb) concentration; and (c) change in peak O_2Hb concentration. Data are presented as the mean ± standard error.

DLPFC-L, left dorsolateral prefrontal cortex (red bars); DLPFC-R, right dorsolateral prefrontal cortex (blue bars); CON, control group; EX, aerobic exercise group; HBO, hyperbaric oxygen therapy group; HBO+EX, combined intervention group. K changes are expressed in arbitrary units (a.u.); mean and peak O_2Hb changes are expressed in mM·mm. *$p < 0.05$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- CONSORT2025Checklist.pdf