

Introduction

Dementia risk accumulates across the lifespan, with its incidence increasing sharply in the ageing population. The 2024 Lancet Commission on Dementia Prevention, Intervention, and Care identifies midlife diabetes as a significant modifiable risk factor for dementia. Chronic hyperglycaemia associated with diabetes has been shown to impair blood–brain barrier (BBB) integrity, leading to cerebrovascular dysfunction and perturbing the brain homeostasis. Mounting evidence further suggests that diabetes increases susceptibility to neurodegenerative disorders, including Alzheimer's disease (AD) and vascular dementia (VaD), primarily through mechanisms involving BBB dysfunction, neurovascular unit impairment, and chronic neuroinflammation. Despite these observations, the molecular and vascular biomarkers linking diabetes-induced BBB dysfunction to dementia remain poorly defined. Therefore, our study aims to investigate the impact of chronic hyperglycaemic exposure on BBB integrity and cerebrovascular function using C57BL/6J and Lepr db/db mouse models, with the goal of identifying potential vascular biomarkers associated with diabetes-related cognitive decline.

Material and methods

Animals: Two mouse models of diabetes were employed in this study: First diabetes was induced in 3 months old C57BL/6J mice by intraperitoneal administration of Streptozotocin (STZ) (60 mg/kg/day) for five consecutive days. Hyperglycaemia was maintained for 30 or 60 days, following which behavioural assessments were performed prior to tissue collection. Blood glucose levels were estimated at 1 week post injection and at the termination of the experiment. In the second model, genetically diabetic Lepr db/db mice (4–5 months of age) and their age-matched wild-type were used. Lepr db/db mice carry a spontaneous homozygous point mutation in the leptin receptor (Lepr), resulting in obesity, insulin resistance, chronic hyperglycaemia, and type 2 diabetes. This model is widely used to investigate diabetes-associated vascular dysfunction, cognitive impairment, and neurodegenerative mechanisms..

Immunohistochemistry (IHC): IHC was performed using 30µm brain sections, which were incubated with anti-CD31 and anti-CD13 markers to identify endothelial cells and pericytes, respectively. DyLight (488) and IgG (594) were used to assess the BBB breaching.

Western blotting: Brain samples were lysed in protein lysis buffer (50nM HEPES pH 7.5, 1% Triton X, 500mM NaCl, 0.1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 10mM MgCl₂, 50mM β-glycerophosphate) supplemented with protease inhibitors cocktail (1x). Protein concentration was determined using the BCA assay. Equal amounts of protein (20 µg) were resolved by SDS-PAGE and transferred onto PVDF membranes. Membranes were blocked with 5% BSA in TBS for 1 h and incubated overnight at 4 °C with primary antibody against ZEB-1 (1:1000), Desmin (1:1000) and vimentin (1:1000). After washing with TBST, membranes were incubated with HRP-conjugated anti-5 mouse/anti-rabbit secondary antibody (1:5000) for 1 h. Protein bands were visualized using ECL Plus reagent and imaged with FluorChem FC3.

Open field test (OFT): Locomotor activity and anxiety-like behaviour were assessed using the open field test. STZ-induced diabetic mice were individually placed in the centre of an open-field arena and allowed to explore freely for 10 min. Animal movement was recorded and analysed to quantify total distance travelled, and average velocity in the centre and peripheral zones.

Rotarod: Motor coordination and balance were assessed using the rotarod test. STZ-induced diabetic mice were placed on an accelerating rotating rod, and the time spent on the rotating rod before falling was recorded over multiple trials. The average time was used to evaluate motor performance.

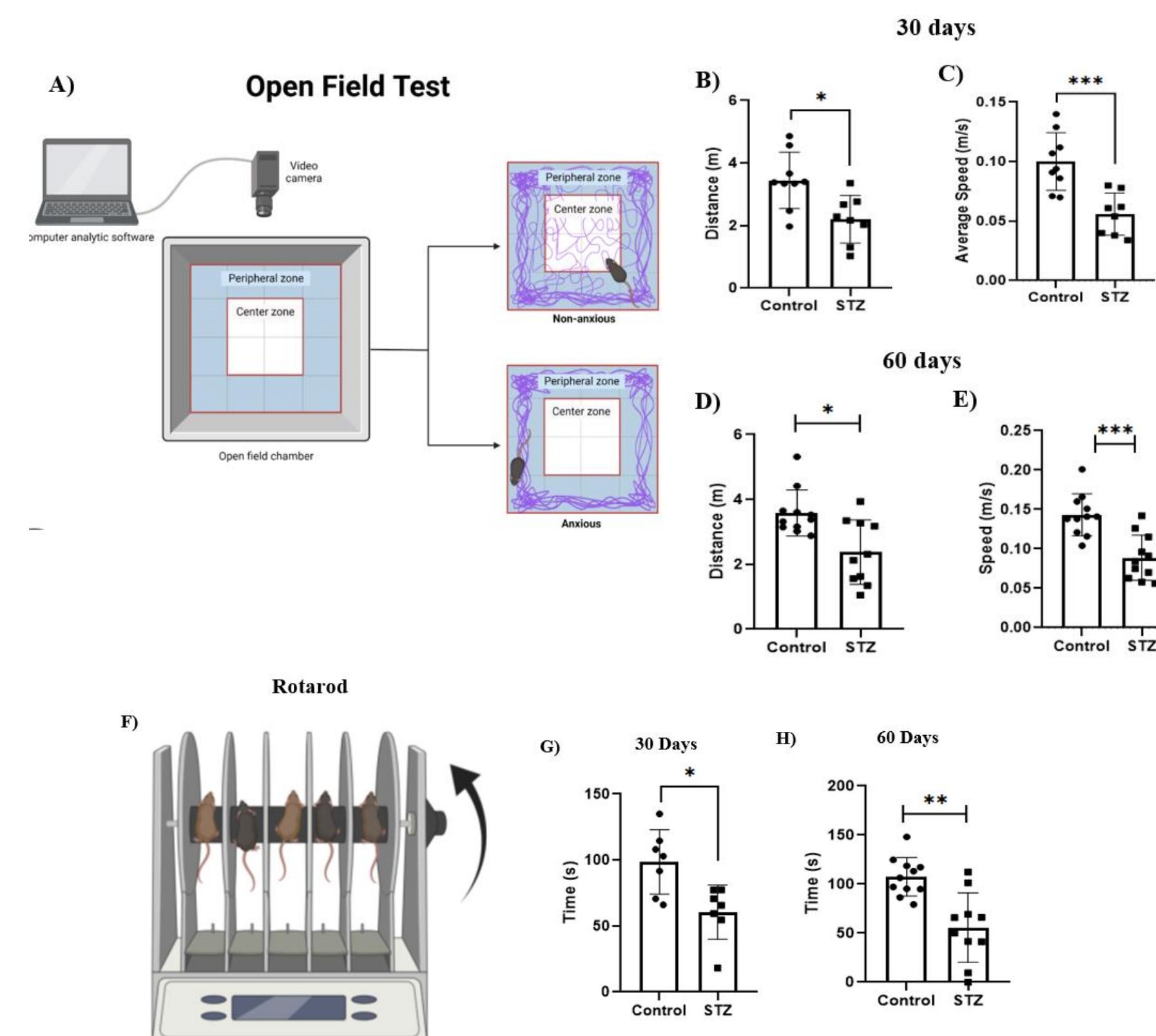


Figure 1: A) Assessment of locomotor activity and motor coordination in STZ-induced diabetic mice. (A) Schematic illustration of the OFT. Graph B) & D) represent the total distance travelled by mice following 30 days (n=8 mice/ group) and 60 days of hyperglycaemia (n=10 mice/group), respectively. Graph C) & E) indicate average speed of mice in the open field after 30 and 60 days of hyperglycaemia, respectively. Data are presented as mean ± SD. (F) Schematic illustration of the rotarod test. Graph G) & H) shows time spent on the rotating rod before falling following 30 (n=7 mice/ group) and 60 days (n=10-11 mice/ group) of hyperglycaemia, respectively. Data are presented as mean ± SD.

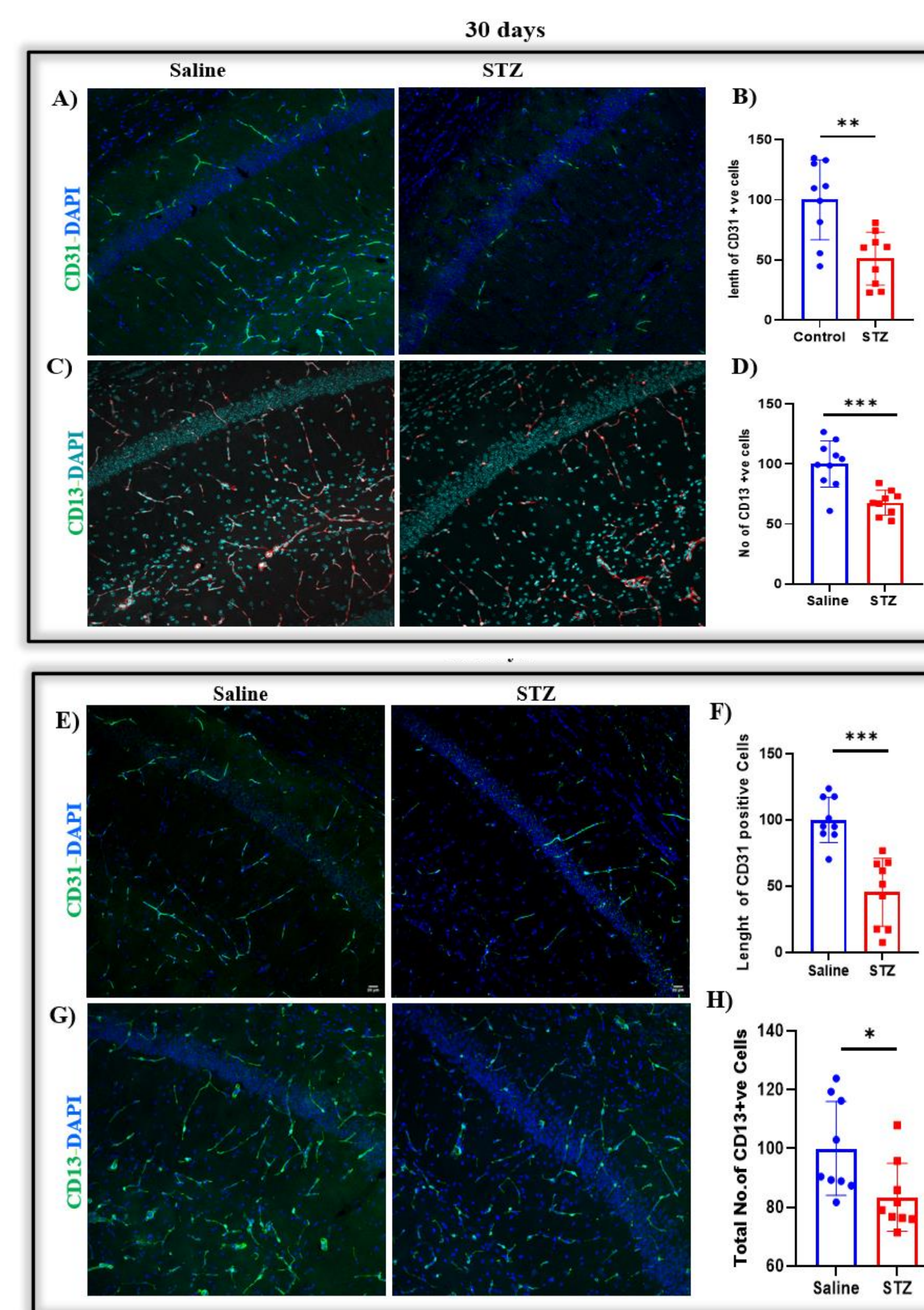


Figure 2: Effect of chronic hyperglycaemia on BBB components. A) Representative images and B) quantification of CD31-positive blood vessel length demonstrating a significant reduction following 30 days of hyperglycaemia. C) Representative images and D) quantification show the decrease in number of CD13-positive pericyte after 30 days of hyperglycaemia. E) Representative images and F) quantification of CD31-positive blood vessel length following 60 days of hyperglycaemia. G) Representative images and H) quantification of CD13-positive pericyte coverage following 60 days of hyperglycaemia. Data were quantified from three brain sections per mouse and are presented as mean ± SD (n = 3 mice per group). Statistical analysis was performed using the Mann–Whitney test.

Results

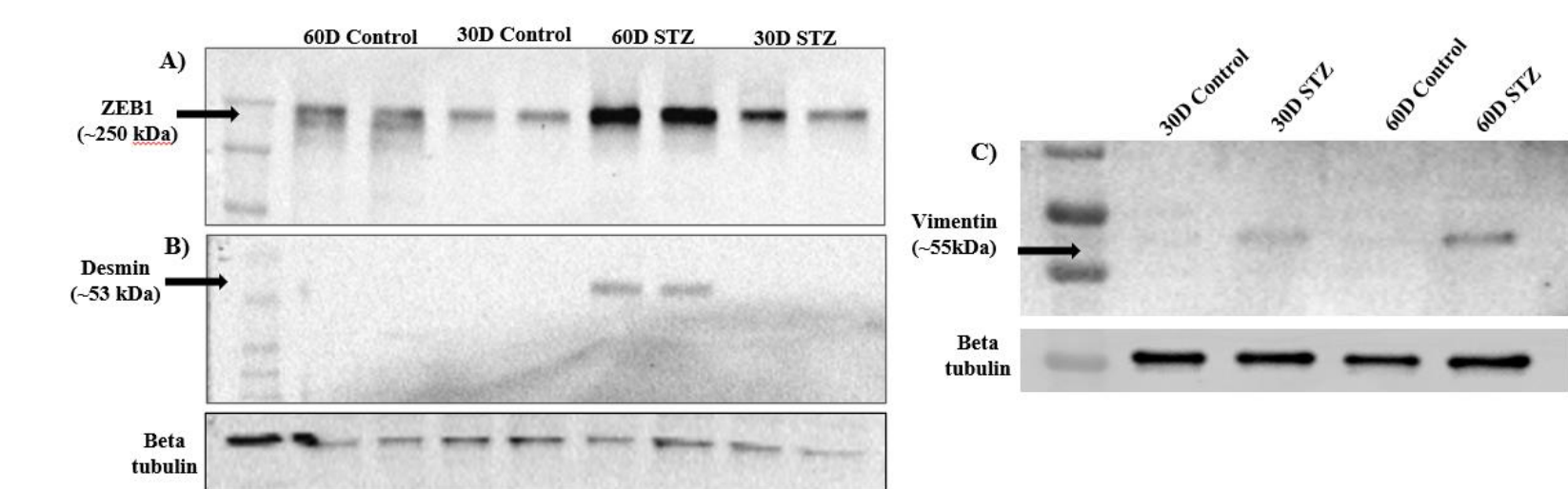


Figure 3: Representative western blot analysis of endothelial-to-mesenchymal transition (EndMT) markers. Representative immunoblots showing the expression of ZEB1, desmin, and vimentin in brain lysates from control and STZ-induced diabetic mice following 30 and 60 days of hyperglycaemia. The blots indicate an increase in the expression of EndMT-associated proteins under chronic hyperglycaemic conditions. Results are representative of two biological replicates (n = 2) for ZEB1 and desmin, and one biological replicate (n = 1) for vimentin.

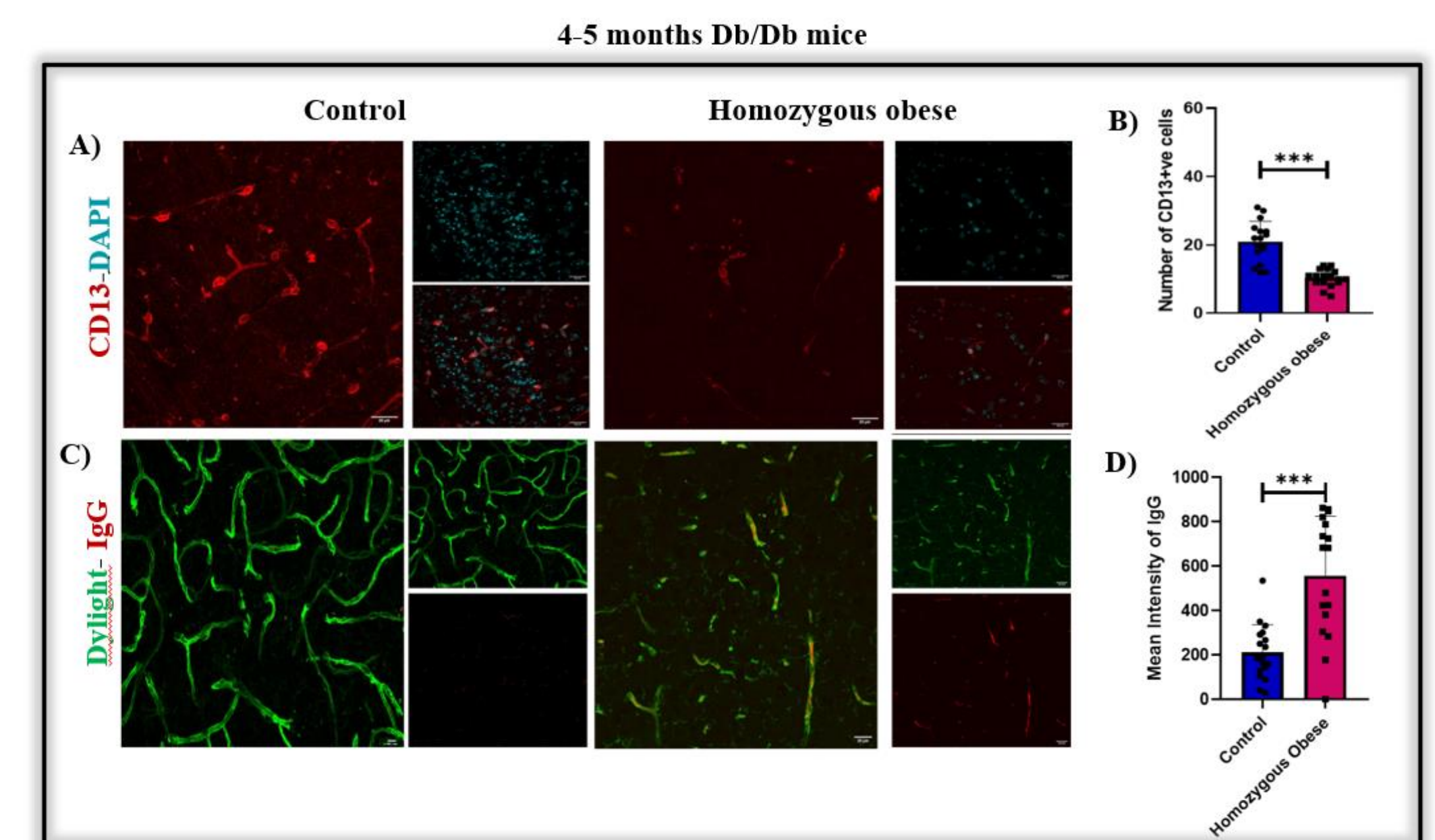


Figure 4: BBB alterations in 4–5-month-old diabetic mice. (A, B) Representative images and quantification of CD13-positive pericytes demonstrating a significant reduction in pericyte number in 4–5-month-old diabetic obese mice compared with age-matched controls. (C, D) Representative images and quantification of mean intensity of IgG shows increased intensity in the brains of 4–5-month-old diabetic obese mice, indicative of BBB disruption. Data were quantified from three brain sections per mouse and are presented as mean ± SD (n = 4 mice per group). Statistical analysis was performed using the Mann–Whitney test.

Conclusion

- Chronic hyperglycaemia impairs locomotor activity and motor coordination in STZ-induced diabetic mice, as demonstrated by the OFT and rotarod performance.
- Prolonged hyperglycaemia disrupts BBB integrity, evidenced by increased IgG extravasation, reduced CD31-positive vessel length, and decreased CD13-positive pericyte number.
- Our data suggests increased expression of endothelial-to-mesenchymal transition (EndMT)-associated proteins such as ZEB1, desmin, and vimentin under chronic hyperglycaemic conditions.
- These findings support a potential role for BBB dysfunction and EndMT in diabetes-associated cerebrovascular pathology and may contribute to the development of dementia.

Acknowledgement

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