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Original Article

Blood-brain barrier integrity disruption is associated with both chronic vascular risk factors and white matter hyperintensities

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ABSTRACT

Background: Cardiovascular risk factors (CRFs) like hypertension, high cholesterol, and diabetes mellitus are increasingly linked to cognitive decline and dementia, especially in cerebral small vessel disease (cSVD). White matter hyperintensities (WMH) are closely associated with cognitive impairment, but the mechanisms behind their development remain unclear. Blood-brain barrier (BBB) dysfunction may be a key factor, particularly in cSVD.

Objective: This study explores the relationship between CRFs, BBB integrity, and WMH burden.

Design, Setting, and Participants: The study included 155 participants from the Biomarkers and Cognition Study, Singapore (BIOCIS). CRFs were assessed through blood tests for glucose and lipid profiles, and blood pressure measurements. WMH volumes were quantified using MRI.

Measurements: BBB integrity was evaluated using a Transendothelial Electrical Resistance (TEER) assay with human brain microvascular endothelial cells (hBMEC) exposed to participant plasma.

Results: Plasma from individuals with a higher WMH burden was associated with increased BBB disruption in hBMEC. Higher systolic and diastolic blood pressure, as well as body mass index, were correlated with greater BBB disruption. Regression analyses revealed that elevated blood glucose and lipid levels were linked to increased BBB disruption. Both periventricular and subcortical WMH burdens were associated with increased BBB disruption.

Conclusion: This study highlights a relationship between CRFs, BBB disruption, and WMH burden, suggesting that CRFs may impair BBB integrity and contribute to WMH and cognitive decline in cSVD.

1. Introduction

Cardiovascular risk factors (CRFs), such as high blood pressure, hyperlipidemia, and diabetes mellitus, are highly prevalent among the elderly population [1,2]. These risk factors significantly contribute to the development of cognitive impairment and dementia [3]. Moreover, CRFs have detrimental cerebral effects in not only dementia and mild cognitive impairment (MCI) but also in cognitively intact elderly individuals, owing to their association with cerebral small vessel disease (cSVD) [4]. Diabetes mellitus, in particular, has been associated with a higher odds ratio for cSVD markers, such as periventricular and subcortical white matter lesions, and lacunar infarcts [5]. Other CRFs, including elevated cholesterol and hypertension, have shown significant positive associations with cSVD burden [6]. cSVD is characterized by structural

Abbreviations: CRF, Cardiovascular Risk Factors; cSVD, Cerebral Small Vessel Disease; WMH, White Matter Hyperintensity; BBB, Blood-Brain Barrier; BIOCIS, Biomarkers and Cognition Study, Singapore; TEER, Transendothelial Electrical Resistance; hBMEC, human brain microvascular endothelial cells; MCI, Mild Cognitive Impairment; MRI, Magnetic Resonance Imaging; MPRAGE, Magnetization Prepared Rapid Gradient Echo; TR, Repetition Time; TE, Time to Echo; TI, Inversion Time; FOV, Field-Of-View; FLAIR, Fluid Attenuated Inversion Recovery; PVH, Periventricular White Matter Hyperintensity; DWMH, Subcortical White Matter Hyperintensity; SPM12, Statistical Parametric Mapping; CAT12, Computational Anatomy Toolbox; DARTEL, Diffeomorphic Anatomic Registration Through Exponentiated Lie algebra algorithm; EDTA, Ethylenediaminetetraacetic acid; EGM™-2, Endothelial Cell Growth Medium-2; HI-FBS, Heat-Inactivated Fetal Bovine Serum; FDR, False Discovery Rate; HBA1C, Glycated Hemoglobin; LDL, Low-Density Lipoprotein; SD, Standard Deviation; BP, Blood Pressure; BMI, Body mass index.

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abnormalities in the small arteries, arterioles, capillaries, and venules of the brain, encompassing a spectrum of intracranial vascular diseases with varied pathologies, clinical presentations, and neuroimaging markers. Notably, cSVD is defined by features such as cerebral microbleeds, lacunar infarcts, enlarged perivascular spaces, and White Matter Hyperintensity (WMH) [7]. Among these, WMH is the most studied neuroimaging marker to classify cSVD extent [8]. WMH can be classified into confluent (coalescing clusters) and punctate (non-confluent) types [9,10]. Confluent WMH is associated with greater cognitive decline and disruption to cerebral connectivity [11,12].

The blood-brain barrier (BBB) is a crucial structure for regulating exchanges between the neural milieu and circulatory system, ensuring neuronal function. It consists of endothelial cells, basement membrane, glial podocytes, and pericytes [13]. BBB-forming endothelial cells are packed with tight junctions and adhesion molecule proteins, regulating diffusion. Influx and efflux transporters facilitate selective uptake of metabolites and waste removal [14]. BBB dysfunction has been implicated in the pathogenesis of neurodegenerative diseases and cSVD [15,16], and is likely an early process in cognitive disorders. Mouse models have demonstrated BBB dysfunction preceding cognitive decline, with accelerated BBB permeability linked to cognitive deficits [17]. Furthermore, dynamic contrast-enhanced magnetic resonance imaging (MRI) studies have shown that individuals with baseline BBB leakage exhibited increased parenchymal diffusivity over two years, particularly around perilesional WMH zones [18].

The association between BBB dysfunction and cognitive impairment is of increasing scientific interest, suggesting a potential role in the exacerbation of cSVD markers on neuroimaging. However, current methodologies for investigating the relationship between BBB dysfunction and WMH in the context of cSVD present several limitations. The majority of studies predominantly utilize imaging-based measures of BBB permeability, such as dynamic contrast-enhanced MRI, which, while informative, lack the specificity to directly assess cellular-level disruptions in endothelial integrity [19,20]. Furthermore, these techniques are limited in their capacity to account for the functional impact of circulating plasma factors on BBB integrity. A limited number of studies has explored how plasma biomarkers associated with CRFs might directly compromise BBB function in experimental systems. Moreover, the majority of research focuses on static measurements of WMH and BBB permeability, often neglecting dynamic interactions between systemic factors such as blood glucose and lipid levels and their influence on WMH progression via BBB disruption. These gaps in knowledge underscore the necessity for experimental models that integrate systemic risk factors and cellular-level BBB assessments to elucidate the mechanistic understanding of WMH development.

To address these gaps, studies have employed transendothelial electrical resistance (TEER) and permeability assays, which are well-established methods for assessing BBB integrity [21,22]. TEER is particularly recognized for its sensitivity in detecting subtle alterations in endothelial barrier permeability. By quantifying electrical resistance across the endothelial monolayer, TEER reflects changes in tight junction integrity, thereby enabling the detection of early disruptions in BBB function. This sensitivity is particularly valuable in studying cSVD, where subtle variations in BBB integrity associated with vascular risk factors can provide significant insights into disease mechanisms [23]. Limited studies, however, have evaluated TEER changes in response to plasma from cSVD patients, a fundamental metric reflecting endothelial barrier integrity [24].

In addition to its sensitivity, TEER demonstrates specificity for paracellular ionic conductance, providing a precise measure of ionic permeability across the endothelial barrier. Alterations in resistance observed in TEER measurements are directly attributable to disruptions in BBB integrity, as the assay specifically quantifies the ionic conductance of the paracellular pathway. Through the utilization of human brain microvascular endothelial cells (hBMECs), which closely approximate the properties of the human BBB, TEER assays enhance specificity for human-relevant BBB characteristics [23]. This approach, which depends on the formation of tight junctions between adherent cells, allows controlled manipulation of experimental conditions to assess BBB integrity [25–27]. These features make the TEER assay a robust and reliable tool for evaluating BBB function under experimental conditions, providing novel insights into the interplay between CRFs, BBB dysfunction, and cSVD pathophysiology.

This study examines the relationship between CRFs, such as blood glucose, blood pressure, and lipid profiles, with BBB integrity quantified via TEER. Additionally, the association between TEER and WMH was studied. We hypothesized a strong association between BBB dysfunction, as measured by TEER, with WMH volumes, and that TEER would correlate with CRFs.

2. Methods

2.1. Participants

One hundred and fifty-five participants from Dementia Research Centre (Singapore) were included in this study. Participants were recruited from the ongoing Biomarkers and Cognition Study, Singapore (BIOCIS) [28]. The inclusion criteria for the BIOCIS study were individuals aged 30 to 95 years from the community with cognitive concerns but with intact mental capacity and without serious neurological, psychiatric, or systemic diseases. The exclusion criteria were illiteracy, clinical diagnosis of serious mental illness, contraindications to Magnetic Resonance Imaging (MRI), diagnosis of cancer within the last two years prior to screening, and pregnancy. Informed consent was obtained from participants according to the Declaration of Helsinki and local clinical research regulations. Furthermore, the procedures utilized in this study were in accordance with ethical guidelines and approved by the Nanyang Technological University Institutional Review Board (study 2021–1036).

2.2. Image acquisition

MRI was performed using a 3T Prisma Fit system (Siemens, Erlangen, Germany). High resolution T1-weighted MPRAGE (Magnetization Prepared Rapid Gradient Echo: 176 continuous sagittal slices, TR/TE/TI = 2000/2.26/800 ms, flip angle = 8°, FOV = 256 × 256mm^2, matrix = 256 × 256, isotropic voxel size = 1.0 × 1.0 × 1.0mm^3, bandwidth = 200 Hz/pixel) and FLAIR (Fluid Attenuated Inversion Recovery: 192 continuous sagittal slices, TR/TE/TI = 7000/394/2100 ms, flip angle = 120°, FOV = 320 × 320mm^2, matrix = 320 × 320, isotropic voxel size = 0.8 × 0.8 × 1.0mm^3, bandwidth = 650 Hz/pixel) sequences were acquired. Scanned images were reviewed during acquisition, and participants with severe motion artifacts and overt pathological findings were excluded.

2.3. Visual ratings of white matter hyperintensities

Two trained raters visually evaluated the FLAIR scans for WMH severity using the modified Fazekas scale, where periventricular WMH (PVH) and subcortical WMH (DWMH) were separately rated on a 0–3 point scale for each hemisphere [9,29]. The scoring criteria were as follows: For PVH, absence of any WMH = 0; presence of caps or pencil-thin lining = 1; smooth halo along the edges of the lateral ventricle = 2; irregular hyperintensities extending into the subcortical white matter = 3. For DWMH, absence of any WMH = 0; presence of nonconfluent foci of WMH in the subcortical region = 1; beginning of confluence of WMH foci = 2; presence of large confluent areas = 3. Discrepancies between the two raters in the MRI visual rating score were resolved by consensus. Subjects were additionally classified as having confluent or nonconfluent WMH based on the Staals criteria [10]: Subjects with a WMH rating of 3 in either periventricular and/or a rating of 2 or 3 in subcor-

tical white matter regions in either hemisphere were assigned as having confluent WMH and the rest as non-confluent WMH.

2.4. Image pre-processing and white matter hyperintensity volume derivation

The Computational Anatomy Toolbox (http://dbm.neuro.uni-jena.de/cat12/) protocol was built into Statistical Parametric Mapping (SPM12) (http://www.fil.ion.ucl.ac.uk/spm/) and utilized to process the T1-weighted images. The normalization of the above-mentioned 3D images used affine transformation and nonlinear registration with correction for bias field inhomogeneities. To obtain better precision in spatial normalization to the standard MNI template, Diffeomorphic Anatomic Registration Through Exponentiated Lie algebra algorithm (DARTEL) was used [30]. A modulation step involving nonlinear deformation on the normalized segmented images was carried out to provide a comparison of the absolute amount of tissue corrected for individual differences in brain size. Finally, all segmented, normalized, and modulated Grey Matter and White Matter images were smoothed with an 8 mm full-width-half-maximum isotropic Gaussian smoothing kernel. To quantify WMH volumes, CAT12 method based on the 3D T1-weighted sequence and region-growing approach comparable to currently used T2/FLAIR-based methods was used [31,32].

2.5. Blood collection, processing and analysis

Before venipuncture, blood pressure was measured to ensure safety and eligibility for blood collection. Subsequently, venous blood was collected from each participant in EDTA Becton Dickinson Vacutainer ® tubes. Plasma was immediately separated through centrifugation (2000 g for 10 min at 4 °C) and refrigerated at −80 °C. Venous blood collected in Serum Separating Tube, EDTA and sodium fluoride tube were sent to an accredited commercial biochemistry laboratory (Innoquest Laboratories, Singapore) within 4 h of the blood draw for blood-based cardiovascular risk analysis.

2.6. Transendothelial electrical resistance (TEER) assay

hBMEC (afirmus bioscience) were seeded in a 24 well transwell® plate (Corning Transwell 3470 Polystyrene Membrane Insert, Sterile, 6.5 mm Membrane Diameter, 0.4μ m Membrane Pore Size, 0.33cm^2 Membrane Area) with a cell density of 3×10^5 cells/cm^2 cultured in (EGM^{TM}-2) Endothelial Cell Growth Medium-2 BulletKit^{TM} (Lonza Bioscience) supplemented with 10% Heat-Inactivated Fetal Bovine Serum (HI-FBS from life technologies) for 8 h. Following which, the hBMECs were starved with EGM^{TM}-2 supplemented with 2% HI-FBS for 2 h before challenging them with collected participant's plasma for 6 h. Finally, TEER measurements were performed using an EVOM2^{TM} Epithelial Voltohmmeter and its STX2 chopstick electrode (World Precision Instruments), following the plasma challenge.

2.7. Statistical analyses

2.7.1. Association of WMH volume with TEER values

A linear regression model was built with TEER as the dependent variable to assess the association between WMH severity and TEER. Natural logarithm transformed WMH volume data was designated as the independent variable and age at visit (stratified as "young" or "old" where "young" was defined as those aged below 65 and old as those aged 65 and above), sex and Total intracranial volume were included as covariates in the model. The statistically significant effect of WMH volume was based on a p-value threshold of $p < 0.05$ after False Discovery Rate (FDR) correction.

To identify differences in TEER between participants with low and high WMH burden, mean TEER values between subjects with confluent and non-confluent WMH were compared using analysis of covariance. Furthermore, the mean TEER values for low and high WMH burden in the periventricular and subcortical white matter regions were also similarly compared. Age and sex were included as covariates in this analysis. Statistical significance between groups was defined based on a $p < 0.05$ threshold after FDR correction.

2.7.2. Association between CRFs and TEER values

To assess the correlation between blood glucose levels, lipid profile, blood pressure and TEER, a linear regression model was built with TEER as the dependent variable and the respective CRF variables (HBA1C, fasting blood glucose, total cholesterol, low-density lipoprotein (LDL), and triglyceride) as the independent variables of interest. Age, sex, and chronic medications used to target cardiovascular risk factors were included as covariates in the linear model. Statistical significance was defined as a p-value threshold of $p < 0.05$ after FDR correction.

All statistical analyses were conducted using R 4.2.3 (R CoreTeam, 2021) with RStudio (RStudio Team, 2023).

3. Results

The age range of the cohort was 31.89 to 84.18 years with a mean of 63.12 (SD = 9.70) years with majority of participants being female (60.0%). Of the 155 participants, 76 (49%) had low TEER defined as values below the mean split of the total TEER value (Table 1). The mean split for TEER value was used to determine low and high TEER, as no standard or published threshold is available for such groupings thus far. Low TEER is indicative of greater transendothelial junction integrity disruption and thus a surrogate measure for the deterioration of BBB integrity disruption.

Participants with low TEER (BBB integrity disruption) had significantly higher WMH volume (5.1 mL vs. 2.8 mL, p = <0.001), significantly higher systolic BP (136.5 mmHg vs 129.1 mmHg, p = 0.00698), significantly higher diastolic BP (81.4 mmHg vs. 77.0 mmHg, p = 0.0163) and significantly higher BMI (24.7kg/m^2 vs 22.8kg/m^2, p = <0.001) compared to participants with high TEER (Table 1). The tryglyceride levels demonstrated a trend towards being higher in the low TEER group.

3.1. Association between WMH and TEER

Based on the linear regression model built for WMH and TEER, we observed that lower TEER values were related to greater WMH volume ($\beta = -1.71$, FDR-corrected $p = 2.41 \times 10^{-3}$) (Fig. 1A and Table 2). This suggests that there is a direct association between increase in WMH volume, and disruption in the barrier integrity of the hBMEC (as observed by the lower TEER). Additionally, TEER measurements were significantly lower in the confluent WMH group than in the non-confluent WMH group (FDR-corrected $p = 6.82 \times 10^{-6}$) (Fig. 1B and Table 2). High periventricular WMH and high subcortical WMH were also associated with significantly lower TEER values in comparison to low WMH burden in the respective regions (FDR-corrected $p = 0.0243$ and FDR-corrected $p = 6.82 \times 10^{-6}$ respectively) (Fig. 1C, Fig. 1D and Table 2).

3.2. Association between blood glucose and TEER

Examination of the relationship between blood glucose levels and TEER indicated that TEER decreased with increasing blood glucose levels. To understand whether long-term or short-term increases in blood glucose levels contribute more to the reduction in TEER value, both HBA1C levels and fasting blood glucose levels were evaluated against TEER. Based on this analysis, we observed that both HBA1C ($\beta = -1.80$, FDR-corrected $p = 0.0147$) and fasting blood glucose levels ($\beta = -1.24$, FDR-corrected $p = 6.70 \times 10^{-3}$) were negatively associated with TEER (Fig. 2A, Fig. 2B and Table 2).

Table 1
Baseline characteristics of participants.

Category	Low TEER ($N = 76$)	High TEER ($N = 79$)	95% Confidence Interval	p-value
Age (years)	64.3 (8.9)	62.0 (10.4)	(−0.783, 5.34)	0.144
Sex (F %)	39 (51.3%)	54 (68.4%)	(0.03, 0.32)	0.0454*
Grey matter volume (mL)	620.8 (65.3)	612.9 (55.2)	(−11.3, 27.2)	0.415
Total intracranial volume (mL)	1464.6 (151.6)	1422.0 (138.9)	(−3.58, 88.8)	0.0703
White matter hyperintensity (mL)	5.1 (4.7)	2.8 (2.9)	(0.993, 3.48)	<0.001*
HBA1C (%)	6.0 (0.8)	5.9 (0.6)	(−0.0768, 0.371)	0.196
Fasting blood glucose (mmol/L)	5.3 (1.1)	5.1 (1.0)	(−0.113, 0.547)	0.195
Total cholesterol (mmol/L)	5.4 (1.1)	5.3 (1.3)	(−0.325, 0.440)	0.768
Low-Density Lipoprotein (mmol/L)	3.1 (1.0)	3.1 (1.1)	(−0.336, 0.312)	0.942
Triglyceride (mmol/L)	1.3 (0.7)	1.1 (0.5)	(−0.0148, 0.376)	0.0698
Systolic blood pressure (mmHg)	136.5 (15.5)	129.1 (18.1)	(2.05, 12.7)	0.00698*
Diastolic blood pressure (mmHg)	81.4 (10.8)	77.0 (11.8)	(0.824, 8.02)	0.0163*
Smoking (%)	7 (9.2)	7 (8.9)	(0, 0.17)	1.000
Body mass index (kg/m^2)	24.7 (3.7)	22.8 (3.2)	(0.816, 2.98)	<0.001*

Values are given as means (SD) unless otherwise stated. Superscript (*) denotes statistically significant differences between the Low and High TEER groups ($p < 0.05$).

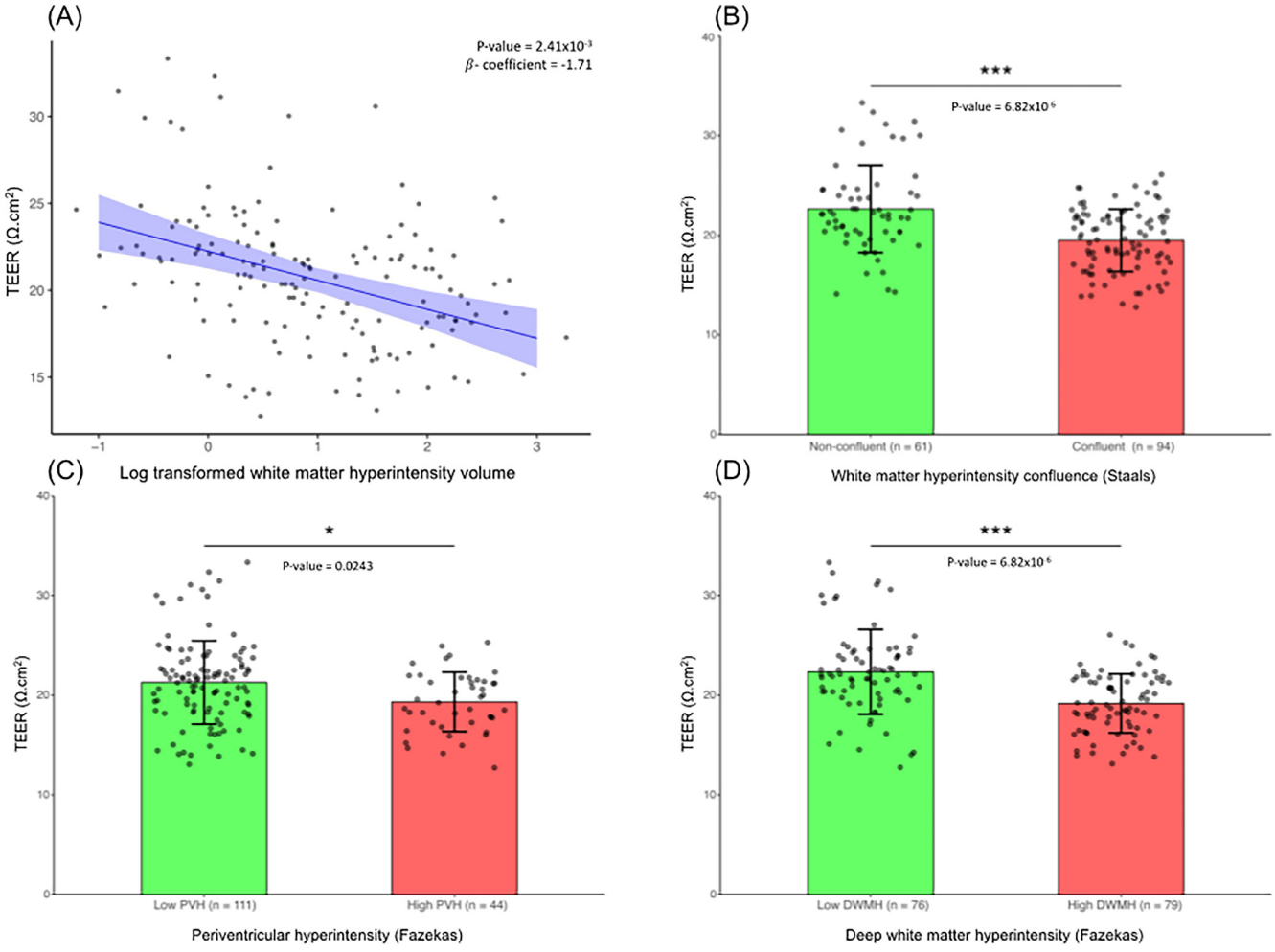

Fig. 1. White matter hyperintensity load is related to reduced transendothelial electrical resistance across human brain microvascular endothelial cells (A). It was observed that individuals presenting with confluent white matter hyperintensity exhibited significantly lower transendothelial resistance values than those with non-confluent white matter hyperintensity, both in the periventricular (C) and subcortical white matter regions (D), following the exposure of human brain microvascular endothelial cells to the participants' plasma (B). Abbreviations: TEER, transendothelial electrical resistance; PVH, periventricular hyperintensity; DWMH, subcortical white matter hyperintensity.

Table 2
Association analysis between TEER and CRFs.

Model	Variable	β-coefficient	Standard error	p-value
TEER ~ HBA1C	HBA1C	−1.80	0.715	0.0180 *
TEER ~ Fasting Blood Glucose	Fasting Blood Glucose	−1.24	0.439	8.19×10^{-3} *
TEER ~ Total Cholesterol	Total Cholesterol	−1.47	0.408	1.54×10^{-3} *
TEER ~ Triglyceride	Triglyceride	−1.89	0.660	8.19×10^{-3} *
TEER ~ Low Density Lipoprotein	Low Density Lipoprotein	−1.42	0.461	5.54×10^{-3} *
TEER ~ Systolic Blood Pressure	Systolic Blood Pressure	−0.0545	0.0302	0.0806
TEER ~ Diastolic Blood Pressure	Diastolic Blood Pressure	−0.0579	0.0370	0.119

Superscript (*) denotes statistically significant differences for the linear regression model after FDR correction ($p < 0.05$). The linear regression analyses presented in this table incorporate adjustments for age, gender, medications administered for CRF management, and the interaction between CRFs and age. Abbreviation: HBA1C, Haemoglobin A1C; FDR, false discovery rate; TEER, transendothelial electrical resistance; CRFs, cardiovascular risk factors.

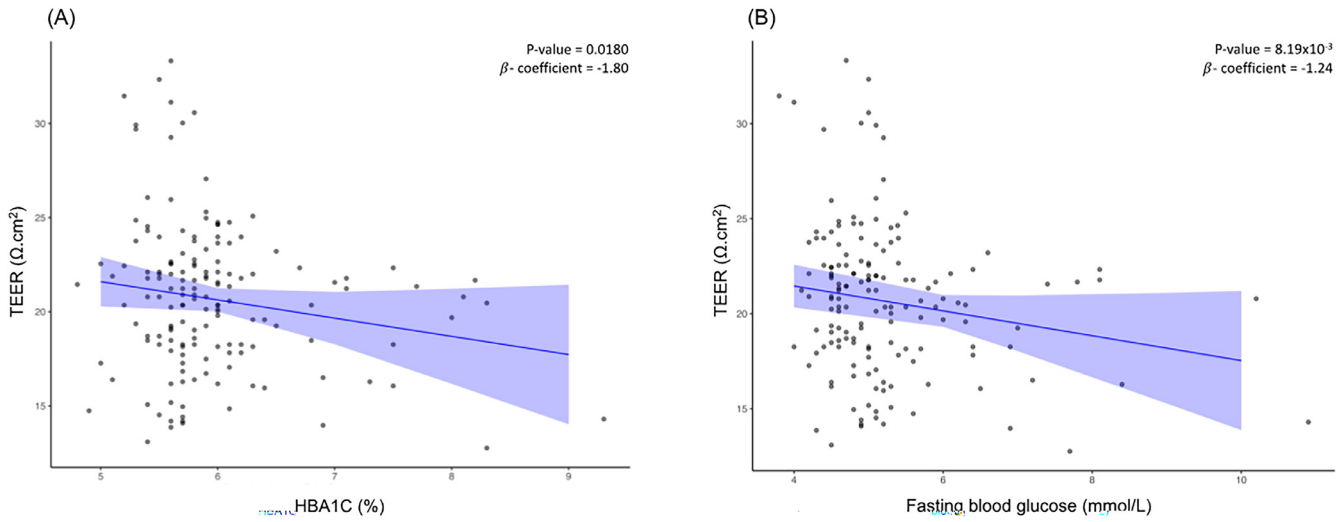

Fig. 2. High blood glucose level was related to a decrease in transendothelial electrical resistance across human brain microvascular endothelial cells, as demonstrated by both the HBA1C marker (A) and fasting blood glucose level (B). Abbreviations: HBA1C, Haemoglobin A1C; TEER, transendothelial electrical resistance.

3.3. Association between blood lipid profile and TEER

Upon assessment of the relationship between blood lipid profile and TEER, higher total cholesterol level was associated with lower TEER ($\beta = -1.47$, FDR-corrected $p = 1.26 \times 10^{-3}$) (Fig. 3A and Table 2). This trend was also observed for triglycerides ($\beta = -1.89$, FDR-corrected $p = 6.70 \times 10^{-3}$) and LDL ($\beta = -1.42$, FDR-corrected $p = 4.53 \times 10^{-3}$) (Fig. 3B, Fig. 3C and Table 2).

3.4. Association between blood pressure and TEER

Upon evaluating the relationship between systolic and diastolic blood pressure with TEER ($\beta = -0.0545$, FDR-corrected $p = 0.0733$ and $\beta = -0.0579$, FDR-corrected $p = 0.119$ respectively) (Fig. 4A, Fig. 4B and Table 2), it was determined that there was no association between the two variables and TEER although participants with low TEER had significantly higher systolic and diastolic BP.

4. Discussion

In this study, we examined the influence of BBB integrity disruption with both WMH and CRF using plasma of individuals with MRI quantified WMH. We demonstrate that the exposure of blood plasma from individuals with high WMH, both in the periventricular and subcortical white matter regions, to be associated with a deterioration in the junction integrity of hBMECs. Furthermore, the higher systolic BP, diastolic BP and BMI in individuals with BBB integrity disruption as well as the observed regression-based association between elevated blood sugar levels and altered lipid profiles with reduced hBMEC TEER value supports our hypothesis that CRFs are strongly associated with the occurrence of BBB integrity disruption.

We have discovered that the blood plasma of individuals with WMH illustrates a detrimental impact on TEER across hBMECs. Specifically, a higher WMH load was found to be correlated with lower TEER, indicating increased BBB junction integrity compromise. Such derogatory associations of WMH on BBB junction integrity are consistent with previous findings of higher BBB permeability in participants with cognitive impairment and cSVD [33,34]. The consistency in the observation, spanning both the periventricular and subcortical white matter regions, may imply that increased BBB integrity disruption is present in regions of both periventricular and subcortical WMH. Hypothesized characteristics of this mechanism include alterations in intercellular structures, decreased expression of transendothelial carriers, the activation of various vasoactive mediators, and the involvement of both astroglia and monocytes/macrophages [35]. It is thus of utmost importance to conduct further longitudinal research to definitively establish the mechanism by which WMH and BBB function are interconnected.

Gender may also influence BBB integrity, potentially mediated by hormonal differences, particularly the role of estrogen [36]. Estrogen has been demonstrated to enhance the expression of tight junction proteins, reduce neuroinflammation, and attenuate oxidative stress, all of which contribute to BBB stability [37]. These protective effects may partially elucidate observed gender differences in BBB function and TEER values, as evidenced by the demographic data (Table 1), which indicate a significantly higher proportion of females in the high TEER group compared to the low TEER group. Females may experience less BBB disruption during premenopausal years due to higher estrogen levels. Conversely, the loss of estrogen in postmenopausal women has been as-

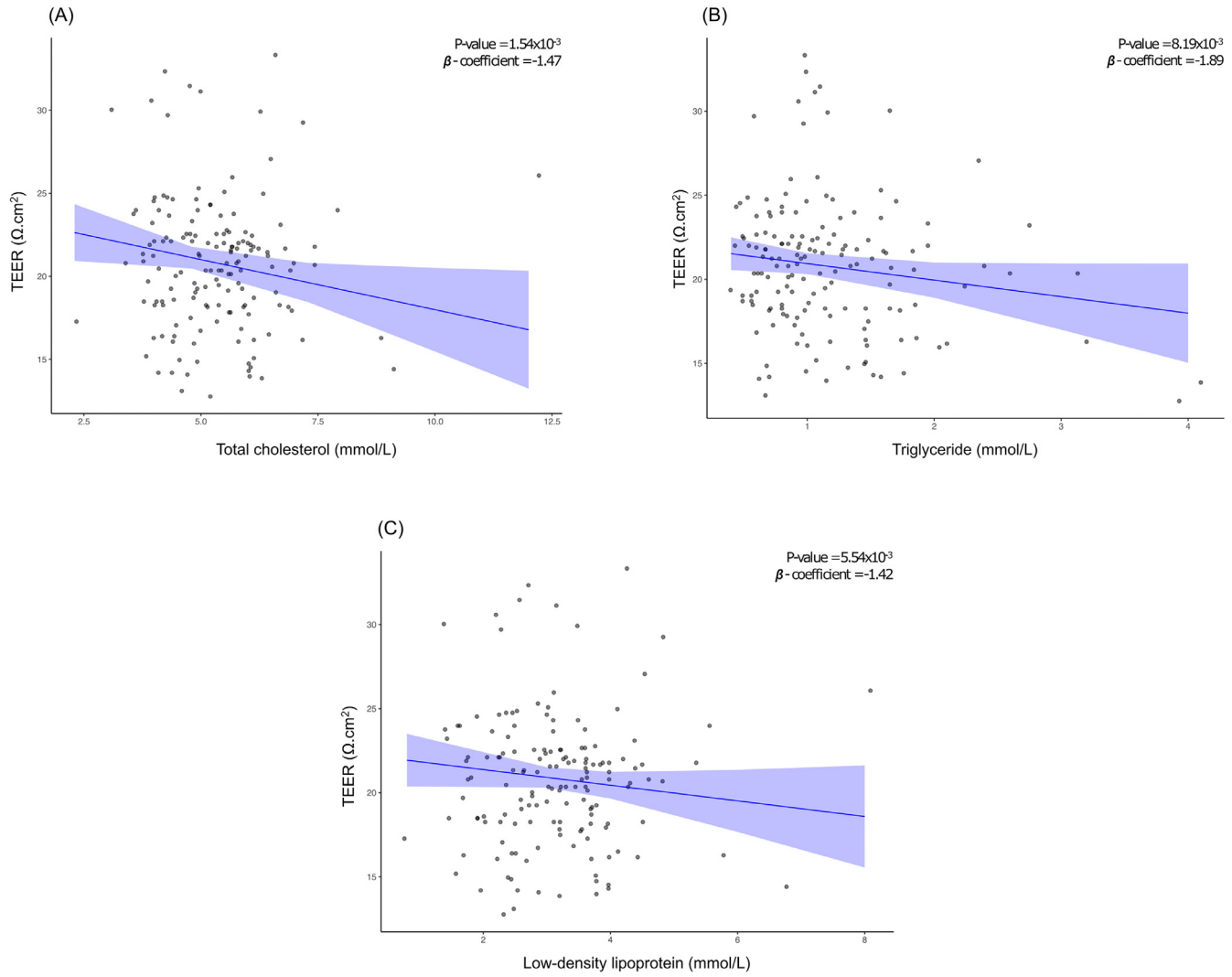

Fig. 3. A significant association was found between elevated lipid levels in the bloodstream and decreased transendothelial electrical resistance across human brain microvascular endothelial cells. This relationship was established through the observed association between higher total cholesterol, triglyceride, and low-density lipoprotein levels, and reduced transendothelial electrical resistance. Abbreviations: TEER, transendothelial electrical resistance.

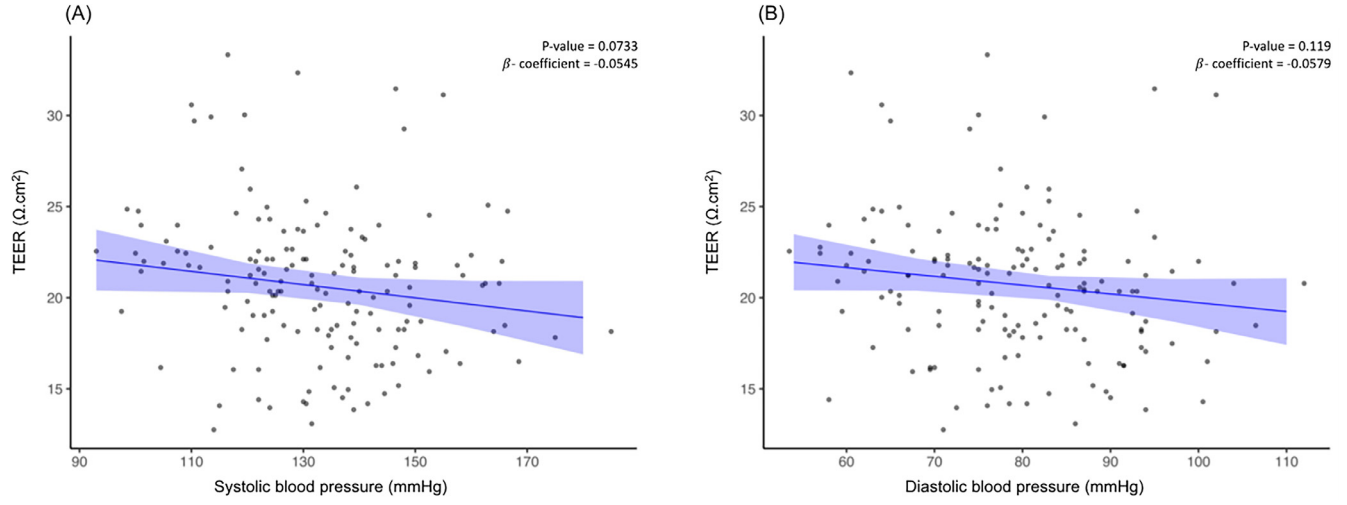

Fig. 4. No significant association was identified between elevated blood pressure and reduced transendothelial electrical resistance across human brain microvascular endothelial cells. This conclusion was drawn on account of the absence of any association between elevated systolic and diastolic blood pressure and diminishing transendothelial electrical resistance. Abbreviation: TEER, transendothelial electrical resistance.

sociated with increased BBB permeability, underscoring its critical role in maintaining BBB integrity [38]. Future investigations should examine how gender and hormonal status interact with other factors such as WMH and CRFs to influence BBB dysfunction.

Comparisons with existing literature elucidate the novelty of our study in utilizing TEER assays to assess BBB integrity in the context of CRFs and cognitive decline. While previous studies, such as Toyama et al. (2018), have explored BBB disruption mechanisms in animal models, these investigations primarily focus on therapeutic approaches like microRNA modulation rather than direct assessments of human plasma effects on BBB integrity [39]. Similarly, Hu et al. (2019) and Inoue et al. (2024) investigate molecular pathways such as apoptosis and histone deacetylase inhibition in BBB dysfunction but do not address human cohorts with cardiovascular risk factors [40,41]. Furthermore, Patel et al. (2017) examines BBB integrity under external insults like HIV-1 Tat protein and methamphetamine exposure, focusing on disease-specific conditions rather than metabolic and vascular influences [42]. These studies underscore the gap in the literature regarding direct assessments of BBB integrity in individuals with CRFs and cognitive decline using TEER assays. By correlating TEER values to plasma from participants with quantified WMH and examining associations with CRFs, our study provides a novel and translational perspective that bridges in vitro findings and human disease relevance, distinguishing it from prior research.

Consistent with previous research, our investigation revealed a positive association between elevated blood glucose levels and BBB junction integrity deterioration [43]. It is postulated that elevated blood glucose levels contribute to increased BBB permeability through the mechanism of metabolic overload and its associated systemic low-grade inflammation. Specific to metabolic overload, it impairs BBB function by increasing paracellular permeability and decreasing TEER. These are changes attributable to the loss of tight junctions, occludin, claudin-5 and vessel basal lamina [43]. Low-grade inflammation can also have a detrimental impact on the function of the BBB by disrupting the balance between matrix metalloproteinases and tissue inhibitors of metalloproteinases (TIMPs) activities. During such prolonged inflammation, matrix metalloproteinases (MMPs) levels increase while TIMPs level decrease, leading to a compromised BBB [43–46]. Prior research has shown that human brain endothelial cells (hCMEC/D3) exhibit lower TEER values when exposed to serum obtained from patients with type 2 diabetes as compared to healthy controls, which is attributed to the elevated levels of tumor necrosis factor - and interleukin-6 in the serum of type 2 diabetes patients [43]. Therefore, it is crucial to conduct additional research into the relationship between metabolic overload, low-grade inflammation, and BBB dysfunction, given the lack of clarity regarding the mechanisms involved. This is particularly important in the context of individuals with elevated glucose levels, as it may shed light on the downstream effects of such conditions on BBB function.

In this current study, we demonstrate the deleterious effects of elevated lipid profile levels on the function of the BBB. Specifically, this was observed for lipid profile constituents such as LDL and triglycerides. Consistent with prior in vivo studies using mice fed with hypercholesterolemic diet, BBB disruption was observed in the prefrontal cortex and hippocampus, and there was a decrease in hippocampal claudin-5 and occludin mRNA levels [47]. Furthermore, it was observed that mice with experimentally induced familial hypercholesterolemia (LDL receptor$^{-/-}$) displayed impaired BBB function when fed a normal diet, and this impairment was further exacerbated when they were fed a high-cholesterol diet [47]. Although the precise mechanism by which LDL impairs BBB function remains unclear, it is well established that the accumulation of LDL in the bloodstream significantly increases the likelihood of LDL oxidation, which in turn triggers oxidative stress and inflammation. Given this information, it has been documented in prior research that in vitro treatment of neural precursor cells with LDL has a profound effect on astrocyte morphology, which is intricately associated with the astrogliosis process. [48]. The neuroinflammatory process is frequently associated with vascular alterations in the brain, including the reconfiguration of microvessels and the entry of circulating cells into the brain parenchyma, which exacerbates neuroinflammation and impairs the BBB [49,50]. Similar to elevated levels of LDL, studies have demonstrated that elevated triglycerides, particularly their lipolysis products, contribute to an increase in the BBB transfer coefficient and induce astrocytic proinflammatory and stress-related gene expression [51–53]. Thus, it is suggested that chronic elevated levels of LDL and triglyceride levels may have indirect consequences on BBB dysfunction. This may be attributed to the accumulation of their metabolic byproducts, which subsequently triggers the activation of neuroinflammation and stress-related genes, ultimately resulting in cell death. Further investigation is necessary in future studies to elucidate the relationship between this phenomenon and BBB dysfunction, as well as its potential contribution to cognitive impairment.

It is perplexing that we did not observe a direct correlation between TEER and both levels of systolic and diastolic BP, although a significantly higher systolic and diastolic BP was observed in individuals and in vivo rodent models with high BBB dyspermeability [54,55]. While we do not have a clear explanation, a potential mechanism for the association between BP and BBB permeability could be the chronicity of elevated BP. Studies have demonstrated that hypertension in mid-life but not late-life to be associated with dementia and hence it may not be the current status of BP but the duration of BP that may influence TEER values [56,57]. Unfortunately, we lack data on duration of elevated BP which hopefully can be addressed in future studies.

Cardiovascular risk factors, including elevated blood glucose and hypercholesterolemia, contribute to BBB integrity disruption through interconnected mechanistic pathways. Hyperglycemia induces metabolic overload in endothelial cells, resulting in oxidative stress and the production of reactive oxygen species. This metabolic disturbance elicits systemic low-grade inflammation, characterized by elevated levels of pro-inflammatory cytokines such as tumor necrosis factor alpha and interleukin-6, which compromise tight junction proteins like occludin and claudin-5, thereby increasing paracellular permeability. Furthermore, hypercholesterolemia exacerbates oxidative stress through the accumulation and oxidation of LDL, which further amplifies neuroinflammation via the activation of astrocytes and microglia. This inflammatory cascade is accompanied by the upregulation of MMPs, particularly MMP-2 and MMP-9, which play a pivotal role in degrading extracellular matrix components, including the basal lamina of the BBB. The increased activity of MMPs contributes to the disruption of endothelial cell anchorage, further compromising BBB structural integrity.

Additionally, MMPs are known to directly cleave tight junction proteins, exacerbating paracellular permeability. Their activity is tightly regulated by TIMPs; however, during chronic inflammation, an imbalance occurs with increased MMP activity and decreased TIMP expression, leading to excessive extracellular matrix remodelling and sustained BBB disruption. Collectively, the combined effects of metabolic overload, systemic inflammation, oxidative stress, and MMP-mediated basement membrane breakdown converge to impair BBB function. This may facilitates the progression of WMH, commonly observed in cSVD (Fig. 5). These intertwined pathways underscore the multifaceted role of CRFs in driving BBB dysfunction and highlight the importance of targeting inflammatory mediators and MMP regulation to mitigate BBB disruption and its downstream pathological effects.

Based on the points discussed above, our study indicates that changes in the components of blood plasma, potentially due to increased blood glucose levels or hyperlipidemia, could contribute to BBB dysfunction, especially in individuals with WMH.

Our study has several limitations. The data utilized in our analyses were cross-sectional and derived from a limited sample size, which warrants further validation through longitudinal datasets and larger sample sizes. Additionally, the inflammatory, amyloid beta, and tau status of the participants remained unknown. Finally, permeability measurement using non-electrolyte tracers could be conducted to further demonstrate the impact of BBB junction integrity on its permeability. Future

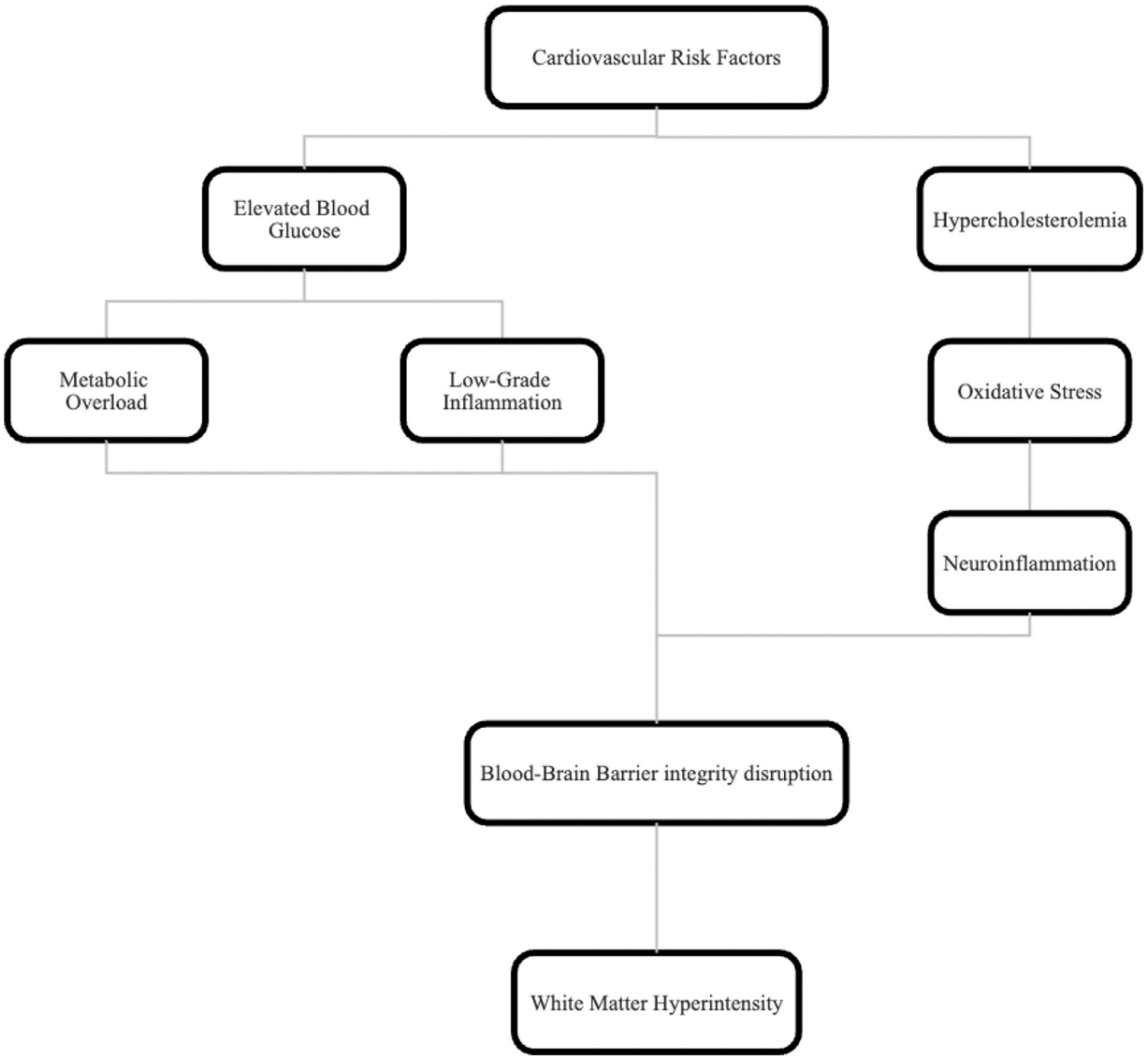

Fig. 5. Flowchart demonstrating the hypothesized relationship between CRFs, BBB integrity and WMH. Abbreviations: CRFs, cardiovascular risk factors; BBB, blood-brain barrier; WMH, white matter hyperintensity.

biomarker studies are expected to provide greater insight into the underlying mechanism of BBB impairment and its implications on the development of WMH and their association with elevated glucose levels and lipid profiles.

5. Conclusion

In summary, the present study provides early evidence that BBB integrity disruption in hBMECs can be induced by exposure to blood plasma from individuals with a high WMH load. Additionally, the study suggests that CRFs specifically elevated glucose levels and lipid profiles may play a significant role in BBB dysfunction, possibly through indirect means. This study underscores the significance of CRFs as precursory warning signs of BBB deterioration, which has been implicated in the development and progression of dementia.

Availability of data and materials

The investigators attest that all data supporting the study's findings are contained within the paper. Additional information and requests for resources and reagents should be communicated with and will be provided by the corresponding author.

Ethics declarations

Ethics approval and consent to participate

The study was approved by the Nanyang Technological University Institutional Review Board (Reference 2021–1036). All participants provided informed consent in accordance with the Declaration of Helsinki and local clinical research regulations.

Consent for publication

Not applicable

Declaration of Generative AI and AI-assisted technologies in the writing process

No generative AI technology was used in the writing process.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

James Xiao Yuan Chen: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ashwati Vipin:** Writing – review & editing. **Gurveen Kaur Sandhu:** Resources. **Yi Jin Leow:** Project administration. **Fatin Zahra Zailan:** Project administration. **Pricilia Tanoto:** Project administration. **Ee Soo Lee:** Writing – review & editing, Methodology. **Khang Leng Lee:** Methodology. **Christine Cheung:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition. **Nagaendran Kandiah:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

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