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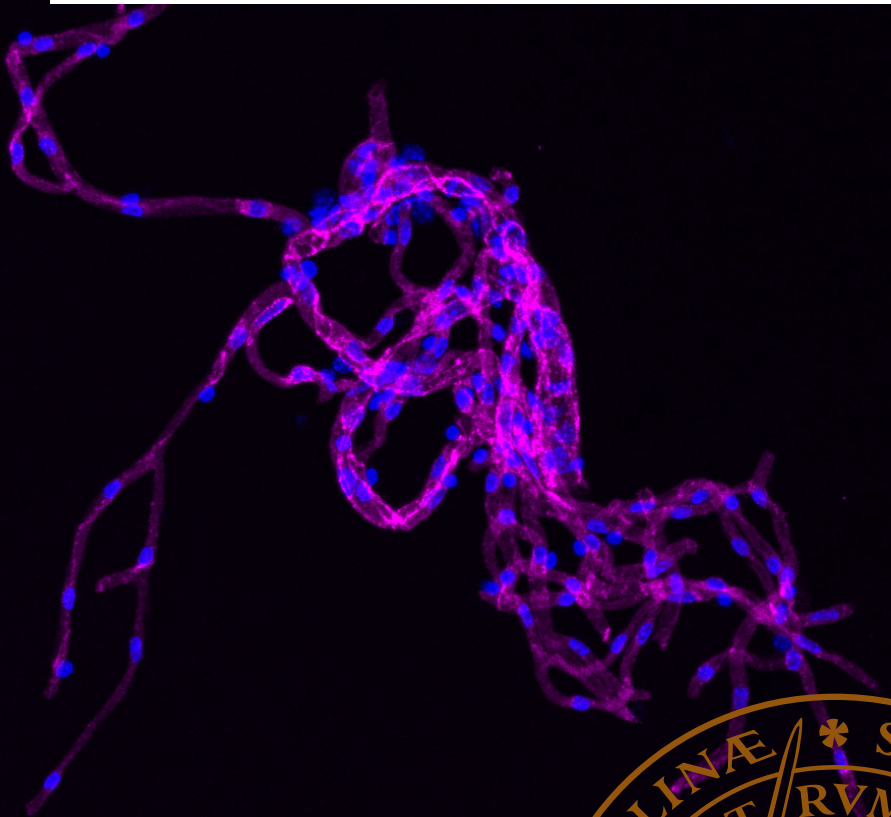
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New Aspects of Sphingosine-1-Phosphate Signaling in Stroke and Obesity

LISA TERESA PORSCHEN

DEPT. OF EXPERIMENTAL MEDICAL SCIENCE | FACULTY OF MEDICINE | LUND UNIVERSITY



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Lisa Teresa Porschen



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DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on September 4th, 2026, at 09.00 in Belfragesalen, Department of Experimental Medical Science, Lund, Sweden.

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Abstract:

Sphingosine-1-phosphate (S1P) regulates multiple processes involved in cerebrovascular function and has emerged as a critical regulator of stroke pathophysiology. Obesity, is a major co-morbidity in stroke; however, the effects of stroke and obesity separately and together on systemic and cerebral S1P signaling, and the therapeutic efficacy of targeting the S1P signaling axis in co-morbid stroke, remain incompletely understood. This thesis investigated the regulation of S1P signaling in diet-induced obesity (DIO) and ischemic stroke.

Acute ischemic stroke reduces circulating S1P levels in both patients and experimental models. S1P depletion is paralleled by increased endothelial activation marker expression, impaired blood-brain barrier integrity, and altered endothelial S1P pathway expression in the ischemic hemisphere, suggesting an important role between S1P depletion and cerebrovascular endothelial alterations. Among S1P receptors (S1PR), S1PR3 emerges as an important post-stroke regulator. Ischemia increases vessel-associated S1PR3 expression, while vessel-associated astrocytes are identified as the main S1PR3-expressing cell population. Both genetic deletion and pharmacological inhibition 4-hours post-stroke reduce infarct volume and improve functional recovery, suggesting S1PR3 as a promising therapeutic target.

Notably, DIO alters several S1P responses relevant to stroke pathology. Specifically, DIO increases circulating S1P levels and alters cerebral S1PR expression with consequences for the post-stroke S1P responses. Thus, DIO attenuates the therapeutic efficacy of S1PR3 antagonism, demonstrating that metabolic status influences stroke-associated S1P responses and treatment efficacy. Since DIO also alters post-stroke S1P levels in the brain, we investigated the effect of DIO on brain S1P signaling and DIO-associated neuroinflammation. DIO induces region-specific alterations of S1P levels, while pharmacological inhibition of S1P generation via sphingosine kinase 2 (SphK2) antagonism attenuates DIO-associated microglial activation and partially restores microglial arborization, implicating SphK2 in obesity-related neuroinflammation.

These findings advance the understanding of S1P signaling in stroke and highlight the importance of considering metabolic context in the development of S1P-targeted stroke therapies.

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Lisa Teresa Porschen



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To my loved ones, who always believed in me

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Abstract

Sphingosine-1-phosphate (S1P) regulates multiple processes involved in cerebrovascular function and has emerged as a critical regulator of stroke pathophysiology. Obesity, is a major co-morbidity in stroke; however, the effects of stroke and obesity separately and together on systemic and cerebral S1P signaling, and the therapeutic efficacy of targeting the S1P signaling axis in co-morbid stroke, remain incompletely understood. This thesis investigated the regulation of S1P signaling in diet-induced obesity (DIO) and ischemic stroke.

Acute ischemic stroke reduces circulating S1P levels in both patients and experimental models. S1P depletion is paralleled by increased endothelial activation marker expression, impaired blood-brain barrier integrity, and altered endothelial S1P pathway expression in the ischemic hemisphere, suggesting an important role between S1P depletion and cerebrovascular endothelial alterations. Among S1P receptors (S1PR), S1PR3 emerges as an important post-stroke regulator. Ischemia increases vessel-associated S1PR3 expression, while vessel-associated astrocytes are identified as the main S1PR3-expressing cell population. Both genetic deletion and pharmacological inhibition 4-hours post-stroke reduce infarct volume and improve functional recovery, suggesting S1PR3 as a promising therapeutic target.

Notably, DIO alters several S1P responses relevant to stroke pathology. Specifically, DIO increases circulating S1P levels and alters cerebral S1PR expression with consequences for the post-stroke S1P responses. Thus, DIO attenuates the therapeutic efficacy of S1PR3 antagonism, demonstrating that metabolic status influences stroke-associated S1P responses and treatment efficacy. Since DIO also alters post-stroke S1P levels in the brain, we investigated the effect of DIO on brain S1P signaling and DIO-associated neuroinflammation. DIO induces region-specific alterations of S1P levels, while pharmacological inhibition of S1P generation via sphingosine kinase 2 (SphK2) antagonism attenuates DIO-associated microglial activation and partially restores microglial arborization, implicating SphK2 in obesity-related neuroinflammation.

These findings advance the understanding of S1P signaling in stroke and highlight the importance of considering metabolic context in the development of S1P-targeted stroke therapies.

Popular Science Summary

A stroke, commonly known as a brain attack, is one of the leading causes of death and long-term disability worldwide. This life-threatening condition happens when the blood supply to part of the brain is interrupted, so brain cells don't get the oxygen and nutrients they need to survive anymore. About 85% of all strokes are caused by a blockage of a blood vessel in the brain. This is known as an ischemic stroke. Without blood flow, brain cells in that area begin to die within minutes. In fact, every hour without treatment causes the brain to lose as many neurons as it normally would during approximately 3.6 years of aging. This is why a stroke is a medical emergency and rapid treatment is crucial. For some patients, emergency surgery can be performed to remove the blockage. In 1996, great hope was raised with the approval of tissue plasminogen activator (tPA) as a treatment for ischemic stroke. This drug dissolves blood clots and thereby restores blood flow in the brain. However, this treatment can only be given within a short time window of up to 4.5 hours after the stroke occurred. Many patients arrive too late to benefit from it. Despite decades of research, treatment options have changed very little since 1996. Then why is it so difficult to find new therapies?

Developing such therapies has proven to be extremely challenging. One reason for this lack of progress is that stroke rarely occurs in otherwise healthy people. Instead most stroke patients are older and often live with other diseases that affect how their brain responds to an injury and recovery. Obesity has become a growing global health problem. It not only increases the risk of having a stroke but also changes how the body reacts after a stroke and how well treatments work. However, experimental studies are traditionally performed in animals that are young and healthy, making it difficult to translate promising findings into effective treatments for patients.

This thesis focusses on the sphingosine-1-phosphate (S1P) system. S1P is a naturally occurring signaling molecule in the body that helps regulate many important processes in the body. After a stroke, however, this system can also contribute to harmful changes in the brain. S1P works by interacting with specific receptors, these are special docking sites on the surface of cells, that, among other functions, help to regulate the body's response after a stroke. By studying how the S1P system changes after stroke, we aimed to better understand its role in ischemic stroke and recovery.

Importantly, we investigated these mechanisms not only in healthy mice but also in obese mice, providing a better representation of the health status of many stroke patients. We found that obesity changes the S1P system response after stroke and reduces the beneficial effects of a therapy that had shown promising results in healthy mice. At the same time, blocking one of the enzymes involved in S1P

production reduced harmful responses in the brain of obese mice, highlighting new possibilities for therapeutic intervention.

Together, these findings provide new insights into how the S1P system is involved in stroke and obesity, both separately and in combination. By taking common patient-related conditions such as obesity into account, this research may contribute to the development of more effective and clinically relevant treatments for stroke in the future.

Populärvetenskaplig Sammanfattning

En stroke, som ofta kallas hjärnslag, är en av de vanligaste orsakerna till dödsfall och långvarig funktionsnedsättning världen över. Detta livshotande tillstånd uppstår när blodtillförseln till en del av hjärnan avbryts, vilket gör att hjärncellerna inte längre får det syre och de näringsämnen de behöver för att överleva. Cirka 85 % av alla strokar orsakas av en blockering i ett blodkärl i hjärnan. Detta kallas för ischemisk stroke. Utan blodflöde börjar hjärncellerna i området att dö inom några minuter. Faktum är att varje timme utan behandling gör att hjärnan förlorar lika många nervceller som den normalt skulle göra under ungefär 3,6 års åldrande. Därför är en stroke ett medicinskt nödläge och snabb behandling är avgörande. För vissa patienter kan akut kirurgi utföras för att avlägsna blockeringen. År 1996 väcktes stora förhoppningar i och med godkännandet av tissue plasminogen activator (tPA) som behandling mot ischemisk stroke. Detta läkemedel löser upp blodproppen och återställer därmed blodflödet i hjärnan. Behandlingen kan dock endast ges inom ett kort tidsfönster på upp till 4,5 timmar efter att stroke inträffat. Många patienter anländer till sjukhuset för sent för att kunna dra nytta av den. Trots årtionden av forskning har behandlingsalternativen förändrats mycket lite sedan 1996. Varför är det då så svårt att hitta nya behandlingar?

Att utveckla sådana behandlingar har visat sig vara extremt utmanande. En orsak till den uteblivna framgången är att en stroke sällan drabbar personer som är i övrigt friska. Istället är de flesta strokepatienter äldre och har ofta andra sjukdomar som påverkar hur deras hjärna reagerar på en skada och återhämtningen efteråt. Fetma är ett växande globalt hälsoproblem. Det ökar inte bara risken för att drabbas av en stroke, utan förändrar också hur kroppen reagerar efter en stroke och hur väl behandlingarna fungerar. Experimentella studier utförs dock traditionellt på unga och friska djur, vilket gör det svårt att omsätta lovande forskningsresultat till effektiva behandlingar för patienter.

Denna avhandling fokuserar på sфingosin-1-fosfat-systemet (S1P). S1P är en naturligt förekommande signalmolekyl i kroppen som bidrar till att reglera många viktiga processer i kroppen. Men efter en stroke kan detta system också bidra till skadliga förändringar i hjärnan. S1P verkar genom att interagera med specifika receptorer, det vill säga speciella bindningsställen på cellernas yta, som bland annat bidrar till att reglera kroppens reaktion efter en stroke. Genom att studera hur S1P-systemet förändras efter en stroke ville vi förstå dess roll vid ischemisk stroke och återhämtning bättre.

Det är viktigt att notera att vi undersökte dessa mekanismer inte bara hos friska möss utan även hos överviktiga möss, vilket mer liknar hälsotillståndet hos många strokepatienter. Vi fann att fetma förändrar S1P-systemets reaktion efter en stroke och minskar de positiva effekterna av en behandling som visat lovande resultat hos friska möss. Samtidigt minskade blockeringen av ett av enzymerna som är

involverat i S1P-produktionen de skadliga reaktionerna i hjärnan hos överviktiga möss, vilket pekar på nya behandlingsmöjligheter.

Sammantaget ger dessa resultat nya insikter i hur S1P-systemet är involverat i stroke och fetma, både var för sig och i kombination. Genom att ta hänsyn till vanliga patientrelaterade tillstånd som fetma kan denna forskning i framtiden bidra till utvecklingen av mer effektiva och kliniskt relevanta behandlingar mot stroke.

Populärwissenschaftliche Zusammenfassung

Ein Schlaganfall, im allgemeinen Sprachgebrauch auch als „Gehirnschlag“ bekannt, ist weltweit eine der häufigsten Ursachen für Tod und langfristige Einschränkungen. Dieser lebensbedrohliche Zustand tritt auf, wenn die Blutversorgung zu einem Teil des Gehirns unterbrochen wird, sodass die Gehirnzellen nicht mehr mit dem Sauerstoff und den Nährstoffen versorgt werden, die sie zum Überleben benötigen. Etwa 85 % aller Schlaganfälle werden durch eine Blockade eines Blutgefäßes im Gehirn verursacht. Dies wird als ischämischer Schlaganfall bezeichnet. Ohne Blutfluss beginnen die Gehirnzellen in diesem Bereich innerhalb weniger Minuten abzusterben. Tatsächlich verliert das Gehirn mit jeder Stunde ohne Behandlung so viele Neuronen, wie es normalerweise während ungefähr 3,6 Jahren des Alterungsprozesses verlieren würde. Deshalb ist ein Schlaganfall ein medizinischer Notfall, und eine schnelle Behandlung ist entscheidend. Bei manchen Patienten kann die blockierte Stelle durch eine Notoperation beseitigt werden. Im Jahr 1996 weckte die Zulassung von tissue plasminogen activator (tPA) zur Behandlung des ischämischen Schlaganfalls große Hoffnungen. Dieses Medikament löst das Blutgerinnsel auf und stellt so den Blutfluss im Gehirn wieder her. Dieses Medikament kann jedoch nur innerhalb eines kurzen Zeitfensters von bis zu 4,5 Stunden nach Eintritt des Schlaganfalls verabreicht werden. Viele Patienten kommen zu spät, um davon zu profitieren. Trotz jahrzehntelanger Forschung haben sich die Behandlungsmöglichkeiten seit 1996 kaum verändert. Warum ist es dann so schwierig, neue Therapien zu finden?

Die Entwicklung solcher Therapien hat sich als äußerst schwierig erwiesen. Ein Grund für diesen ausbleibenden Fortschritt könnte darin liegen, dass ein Schlaganfall bei ansonsten gesunden Menschen eher selten auftritt. Stattdessen sind viele Schlaganfallpatienten älter und leiden häufig unter weiteren Erkrankungen, die beeinflussen, wie ihr Gehirn auf den Schlaganfall reagiert und sich davon erholt. Übergewicht ist zu einem weltweit zunehmenden Gesundheitsproblem geworden. Es erhöht nicht nur das Risiko, einen Schlaganfall zu bekommen, sondern kann auch die Reaktion des Körpers nach einem Schlaganfall und die Wirksamkeit von Therapien verändern. Experimentelle Studien werden jedoch überwiegend an jungen und gesunden Tieren durchgeführt, was es schwierig macht, vielversprechende Erkenntnisse in wirksame Behandlungen für Patienten umzusetzen.

Im Zentrum dieser Doktorarbeit steht das Sphingosin-1-Phosphat (S1P) System. S1P ist ein im Körper natürlich vorkommendes Signalmolekül, das zur Regulierung vieler wichtiger Prozesse im Körper beiträgt. Nach einem Schlaganfall kann dieses System jedoch auch zu schädlichen Veränderungen im Gehirn beitragen. S1P wirkt durch die Interaktion mit spezifischen Rezeptoren, das sind spezielle Bindungsstellen auf der Oberfläche von Zellen, die unter anderem dazu beitragen, die Reaktion des Körpers auf einen Schlaganfall zu regulieren. Durch die

Untersuchung der Veränderungen des S1P-Systems nach einem Schlaganfall wollten wir dessen Rolle bei ischämischen Schlaganfällen und der Genesung besser verstehen.

Wir haben diese System nicht nur bei gesunden Mäusen, sondern auch bei übergewichtigen Mäusen untersucht, wodurch wir den Gesundheitszustand vieler Schlaganfallpatienten besser abbilden konnten. Wir stellten fest, dass Übergewicht die Reaktion des S1P Systems nach einem Schlaganfall verändert und die positiven Wirkungen einer Therapie abschwächt, die bei gesunden Mäusen vielversprechende Ergebnisse zeigte. Gleichzeitig führte die Hemmung eines der an der S1P-Produktion beteiligten Enzym zu einer Verringerung schädlicher Reaktionen im Gehirn von übergewichtigen Mäusen, was neue Möglichkeiten für therapeutische Maßnahmen aufzeigt.

Diese Erkenntnisse liefern insgesamt neue Einblicke darin, wie das S1P System sowohl einzeln als auch in Kombination bei Schlaganfall und Übergewicht eine Rolle spielt. Durch die Berücksichtigung häufiger Begleiterkrankungen wie Übergewicht könnte diese Forschung in Zukunft zur Entwicklung wirksamerer und klinisch relevanterer Behandlungsmethoden für Schlaganfälle beitragen.

Papers Included in the Thesis

Paper I

Porschen LT, Matthes F, Matuskova H, Braadt L, Petzold GC, Lindgren AG, Meissner A. Circulating sphingosine-1-phosphate depletion is associated with endothelial activation and altered brain-endothelial S1P pathway expression in ischemic stroke. *Fluids Barriers CNS* 23, 82 (2026).

Paper II

Matuskova H, **Porschen LT**, Matthes F, Lindgren AG, Petzold GC, Meissner A. Spatiotemporal sphingosine-1-phosphate receptor 3 expression within the cerebral vasculature after ischemic stroke. *Iscience*. 2024 Jun 21;27(6).

Paper III

Vanherle L*, Skoug C*, **Porschen LT**, Gimeno-Ferrer F, Matthes F, Duarte JMN[#], Meissner A[#]. Sphingosine kinase 2 inhibition ameliorates neuroinflammation in diet-induced obese mice. *Aging and disease*. 2025.

Paper IV

Porschen LT, Matthes F, Duarte JMN, Meissner A. Diet-induced obesity alters post-stroke sphingosine-1-phosphate signaling and attenuates therapeutic responsiveness to S1PR3 antagonism. *Manuscript*, 2026.

*[#] These authors contributed equally to this work.

Papers not Included in the Thesis

Skoug C*, Vanherle L*, **Porschen LT**, Fryklund C, Stenkula KG, Meissner A#, Duarte JM#. Subcutaneous administration of the sphingosine kinase 2 inhibitor ABC294640 has no metabolic benefits in high fat diet-induced obesity in male mice. *Life Sciences*. 2025 Sep 4;123952.

Review

Gimeno-Ferrer F, **Porschen LT**, Matthes F, Gohlsch K, Meissner A. Airborne particulates and brain health: The role of PM2. 5 in blood-brain-barrier dysfunction. *Journal of Cerebral Blood Flow & Metabolism*. 2026 May;46(5):1025-42.

*# These authors contributed equally to this work.

Abbreviations

ATP	adenosine triphosphate
BBB	blood-brain barrier
BMI	body mass index
BSA	bovine serum albumin
CBF	cerebral blood flow
CD	control diet
CNS	central nervous system
contra	contralateral
DEXA	dual-energy X-ray absorptiometry
DIO	diet-induced obesity
HDL	high-density lipoprotein
HFD	high-fat diet
ipsi	ipsilateral
MCA	middle cerebral artery
MCAo	middle cerebral artery occlusion
MMP	matrix metalloproteinases
MRI	magnetic resonance imaging
NIHSS	national institutes of health stroke scale
NVU	neurovascular unit
pMCAo	permanent middle cerebral artery occlusion
RARE	rapid imaging with refocused echoes
RCD	rodent chow diet
ROS	reactive oxygen species
S1P	sphingosine-1-phosphate
S1PR/S1pr	sphingosine-1-phosphate receptor
SphK/Sphk	sphingosine kinase
SPNS2	spinster homolog 2
T2D	type 2 diabetes

tMCAo	transient middle cerebral artery occlusion
tPA	tissue plasminogen activator
TTC	2,3,5-triphenyltetrazolium chloride
VEGF	vascular endothelial growth factor
WT	wildtype

Introduction

Ischemic Stroke

Stroke is the second leading cause of death and third leading cause of death and disability combined worldwide (Feigin et al., 2025). The global burden of stroke is expected to rise within the next years, indicating that by 2050, stroke-related deaths and associated costs will increase by more than 50%. This is largely driven by population growth and aging (Feigin et al., 2023), highlighting stroke as a major public health challenge.

Stroke Pathology

Stroke can either be classified into ischemic or hemorrhagic stroke based on its origin (Feigin et al., 2025). Hemorrhagic stroke is caused by a rupture of a blood vessel. Ischemic stroke represents the most common subtype and accounts for approximately 65% of all stroke cases globally and up to 87% in high-income countries (Krishnamurthi et al., 2020; Virani et al., 2020). It is caused by the occlusion of a blood vessel, typically due to a thrombus or blood clot, leading to an interruption in blood flow to the brain tissue (Vandelanotte & De Meyer, 2024). As a consequence, affected brain regions experience rapid deprivation of oxygen and nutrients (Salaudeen et al., 2024). The resulting neurological deficits in patients depend largely on the location and extent of the lesion, including motor, sensory, and cognitive impairment (Gottesman & Hillis, 2010; Siegel et al., 2022).

The pathophysiology of ischemic stroke is complex and involves a cascade of events, including excitotoxicity, oxidative stress, neuroinflammation, apoptosis (Broughton et al., 2009), and autophagy (Qin et al., 2022; Wang et al., 2018). Collectively, these mechanisms contribute to progressive tissue damage and neurological deficits.

Within minutes of the vascular occlusion, irreversible cell death occurs in the ischemic core. Surrounding this core is the so-called penumbra, a region of functionally impaired but potentially salvageable tissue. The penumbra represents a critical therapeutic target region, as timely intervention can restore perfusion and prevent further damage.

Treatment of stroke involves three major approaches: preventive, management, and post-stroke rehabilitation measures (Kuriakose & Xiao, 2020). Currently, the only approved pharmacological treatment for acute ischemic stroke is thrombolysis using tissue plasminogen activator (tPA). While effective in restoring blood flow, its value in clinical practice is limited by a narrow administration window of 3 - 4.5 hours after stroke onset, strict eligibility criteria, and the risk of hemorrhagic transformation (Berge et al., 2021; Kùlkens & Hacke, 2007; Powers et al., 2019). Delayed tPA treatment can exacerbate matrix metalloproteinase (MMP)-mediated blood-brain barrier (BBB) disruption, oxidative stress, and microglia-driven neuroinflammation, thereby contributing to secondary brain injury (Jickling et al., 2014; Lakhan et al., 2013). Consequently, only around 16% of patients are eligible for this treatment and of those eligible, around 60% actually receive it (Ko et al., 2025). Nonetheless, reperfusion of the ischemic penumbra remains the primary goal of acute stroke therapy, as it can reduce infarct size and improve functional outcomes.

Neurovascular Unit in Health and Ischemic Stroke

The central nervous system (CNS) relies on a highly specialized and tightly regulated microenvironment to maintain neuronal function and brain homeostasis. This regulation is achieved by the neurovascular unit (NVU), consisting of multiple components, including neurons, glial cells (astrocytes, microglia, oligodendrocytes), vascular cells (endothelial cells, smooth muscle cells, pericytes), and the basement membrane (Iadecola, 2017; Lo & Rosenberg, 2009) (**Fig. 1**). The concept of the NVU has first been introduced in 2001, emphasizing the intricate communication between brain cells and vascular components that are essential for normal neurological function (Iadecola, 2017).

A central component of the NVU is the BBB, which is a specialized, semi-permeable border between the systemic circulation and the brain parenchyma. The BBB consists of endothelial cells, pericytes, astrocytes, neurons, and the extracellular matrix. The BBB is formed by tightly connected endothelial cells through tight junction proteins, including claudins, occludin, and zonula occludens protein, thereby restricting blood-borne substances from entering the brain (**Fig. 1**). Pericytes are embedded within the basement membrane maintaining vascular stability and BBB integrity. Astrocytic endfeet ensheath the microvasculature and contribute to BBB formation, maintenance and maturation. Microglia, the resident immune cells of the CNS and perivascular macrophages continuously monitor the brain environment and contribute to immune surveillance, tissue repair, and inflammatory responses (Abbott, 2013; Daneman & Prat, 2015). Under physiological conditions, the highly organized cellular architecture of the NVU maintains CNS homeostasis by regulating the exchange of ions, metabolites, and

signaling molecules across the BBB, while simultaneously protecting neural tissue from toxins, pathogens, and peripheral immune cells.

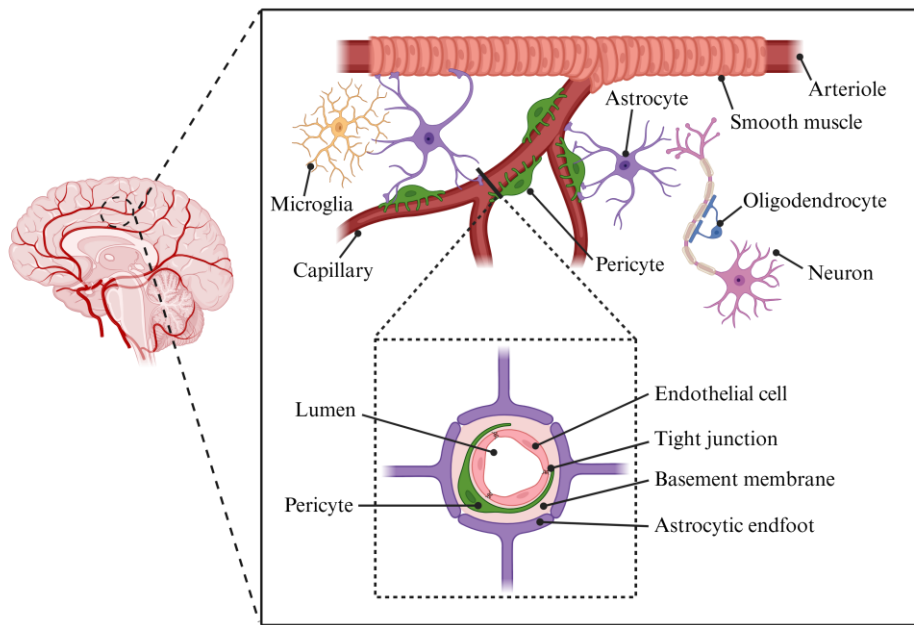


Figure 1| Structure of the neurovascular unit and blood-brain barrier.

Schematic representation of the neurovascular unit (NVU), illustrating the close interaction between neuronal, glial and, vascular compartments. Neurons communicate with cerebral blood vessels and other cellular components of the NVU. Endothelial cells form the inner lining of cerebral blood vessels and are connected by tight junction proteins, which contribute to blood-brain barrier (BBB) integrity. These endothelial cells are surrounded by a basement membrane, within which pericytes are embedded. Within the brain parenchyma, astrocytes extend their endfeet to ensheath the capillary wall, establishing close interactions with both endothelial cells and pericytes. Created with Biorender.com.

The critical function and the tightly coordinated interactions of the NVU are disrupted in response to ischemic stroke. Vessel occlusion leads to an interruption of cerebral blood flow (CBF), resulting in insufficient oxygen and nutrient delivery to the affected brain tissue. The resulting energy failure rapidly disrupts cellular homeostasis, leading to neuronal dysfunction and initiating a cascade of molecular and cellular events that involve all components of the NVU. Within minutes of ischemic injury, adenosine triphosphate (ATP) depletion impairs ion pump activity, leading to membrane depolarization, excessive glutamate release, ionic imbalance (calcium overload), and lactate acidosis resulting into cell death in the ischemic core (Doyle et al., 2008; Xing et al., 2012). Excessive reactive oxygen species (ROS) production induces oxidative stress, contributing to BBB disruption through endothelial injury and loss of barrier integrity (Rodrigo et al., 2013). Endothelial

dysfunction subsequently promotes dysregulated vascular tone, increased vascular permeability and inflammatory signaling (Kleeberg et al., 2025). Endothelial-derived MMPs, in particular MMP9, contribute to degradation of tight junction proteins and thereby further destabilize the barrier (Bauer et al., 2010; Rosenberg & Yang, 2007). Pericytes also undergo a dynamic functional shift during ischemic injury. In the acute phase, they detach from the vessel wall and adopt a contractile and pro-inflammatory phenotype that promotes BBB breakdown through the release of cytokines and MMPs, resulting in impaired microvascular perfusion and increased neuroinflammation. In later stages, pericytes contribute to vascular and neural repair by supporting endothelial regeneration, enhancing vascular endothelial growth factor (VEGF)-mediated angiogenesis, and facilitating neurogenesis and tissue remodeling (Seyedaghamiri et al., 2024; Yang et al., 2017). Astrocytes play a dynamic and context-dependent role. During the acute phase, reactive astrocytes can exacerbate injury through the release of pro-inflammatory and cytotoxic mediators. However, astrocytes also contribute to tissue repair by secreting neurotrophic factors and anti-inflammatory cytokines (Han et al., 2022; Liu & Chopp, 2016). Astrocytes play a particularly important role in the response to ischemic injury by forming the glial scar, which helps contain inflammation, restrict the spread of tissue damage, and establish a protective boundary around the ischemic core (Sofroniew, 2015). Thus, astrocytes serve as critical regulators of BBB integrity and CNS homeostasis, exerting both protective and detrimental effects depending on the stage of injury. Microglia are among the first immune cells to respond to ischemic injury and play a central role in neurovascular regulation. During the acute phase, activated microglia contribute to BBB disruption and neuronal damage through the release of pro-inflammatory cytokines, chemokines, ROS, and other cytotoxic mediators. During later stages, they facilitate tissue repair, debris clearance, and the resolution of inflammation (Zhao et al., 2017). Their ability to switch between detrimental and protective functions underlines their importance in BBB function.

This highlights the integrated and highly dynamic function of the NVU in ischemic stroke. A deeper understanding of these interactions is essential for the development of effective therapies to enhance post-stroke recovery.

Lack of Translational Success to the Clinic

Despite extensive research efforts, the development of effective neuroprotective therapies for ischemic stroke remains limited. While several neuroprotective therapies have shown promising results in experimental studies, their translation into clinical success has not been successful (Sena et al., 2007). Several factors might influence the discrepancy between experimental studies and clinical outcomes.

One major limitation of preclinical stroke research lies in the use of simplified experimental models, which predominantly use young, metabolically healthy male rodents. These animal models fail to adequately reflect the patient's situation in the clinic, where patients often present with advanced age and comorbidities, such as type 2 diabetes (T2D), hypertension, or obesity (Feigin et al., 2024; Fluri et al., 2015). The absence of these common comorbid conditions may majorly influence treatment responses and reduce the translational relevance of preclinical findings.

In addition, methodological differences between preclinical studies and clinical practice further contribute to this gap. Experimental studies frequently administer therapeutic interventions immediately or within minutes after stroke onset or reperfusion, whereas in the clinical setting, treatment is typically initiated hours later due to delays in symptom recognition and hospitalization. This discrepancy is particularly critical given that the ischemic penumbra exists only for a limited time window, up to approximately 12 hours after stroke onset in humans (Paciaroni et al., 2009). Delayed treatment therefore reduces the opportunity to prevent the progressive expansion from the infarct core into the surrounding tissue (Yang & Liu, 2021).

Other contributing factors include differences in drug dosage, pharmacokinetics, and routes of administration between animal studies and human trials. Together, these limitations highlight the need for more clinically relevant preclinical models that incorporate common comorbidities, realistic therapeutic time windows, and optimized treatment paradigms. Addressing these challenges will be essential to improve the translational success of neuroprotective strategies in stroke.

Risk Factors for Stroke

Stroke is influenced by both modifiable and non-modifiable risk factors. Modifiable risk factors include hypertension, tobacco use, diabetes, overweight and obesity and unhealthy diet. Non-modifiable risk factors include age, sex, race, a prior stroke, and certain environmental exposures (**Fig. 2**) (Feigin et al., 2024). Beyond these established risk factors, environmental factors are increasingly recognized as contributors to stroke risk. Growing evidence suggests that exposure to environmental pollutants, particularly airborne particulate matter resulting from industrialization and urbanization, can promote cardiovascular disease, including stroke (Gimeno-Ferrer et al., 2026; O'Donnell et al., 2011). Together, the current research highlights that stroke risk arises from a complex interplay of genetic, environmental, and lifestyle-related factors. Among these, obesity and metabolic syndrome have emerged as major global health challenges due to their increasing prevalence and association with cardiovascular disease.

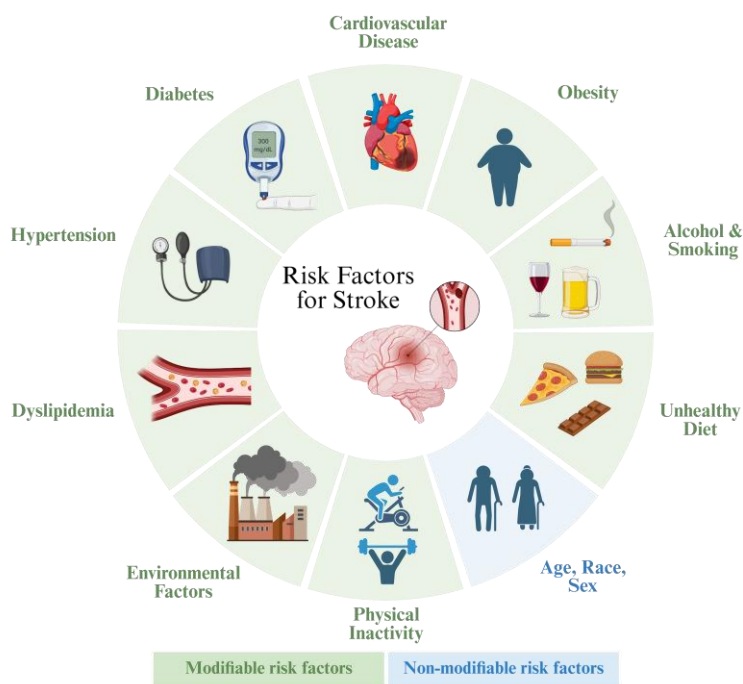


Figure 2| Modifiable and non-modifiable risk factors for stroke.

Schematic overview of the major risk factors associated with stroke. Non-modifiable risk factors include age, sex, and race/ethnicity. Modifiable risk factors include hypertension, dyslipidemia, diabetes mellitus, cardiovascular disease, obesity, unhealthy diet, physical inactivity, smoking, excessive alcohol consumption, and environmental factors. While non-modifiable risk factors cannot be altered, modifiable risk factors can be managed through lifestyle changes and medical interventions. Created with Biorender.com.

Obesity and Metabolic Syndrome

Overweight is defined as a body mass index (BMI) of 25 kg/m^2 or higher, while obesity is defined as a BMI of 30 kg/m^2 or higher. The global prevalence of obesity has increased over recent decades, with approximately 16% of adults classified as obese in 2022. Obesity is a complex, multifactorial chronic disease resulting from a combination of genetic, environmental, and psychosocial determinants and lifestyle factors (World Health Organization, 2022).

Obesity is a risk factor for ischemic stroke (Winter et al., 2008) and also contributes to the development of several risk factors for stroke, including hypertension, diabetes, and dyslipidemia. More broadly, obesity is associated with an increased risk of cardiovascular disease and a range of several related metabolic and vascular abnormalities, including atherosclerosis, hypertension, dyslipidemia, insulin resistance, endothelial dysfunction, and chronic low-grade inflammation (Ford, 2005; Henning, 2021; Kawai et al., 2021; McFarlane et al., 2001; Sowers, 2003).

These associations underline the potential systemic impact of obesity on vascular and metabolic homeostasis and ultimately stroke risk.

Experimental studies further support the role of obesity in stroke. In genetic models, such as leptin- or leptin receptor-deficient mice (ob/ob and db/db mice, as well as Zucker rats), obesity leads to worsened stroke outcome (Haley & Lawrence, 2016). Similarly, diet-induced obesity (DIO) models show increased infarct size, BBB disruption, neuroinflammation, and a higher incidence of hemorrhagic transformation (Grisotto et al., 2021; Peterson et al., 2021; Terao et al., 2008). Several mechanisms may contribute to these observations, including endothelial dysfunction and increased arterial stiffness, which impair vascular reactivity and collateral circulation. These vascular alterations can promote expansion of ischemic injury from the ischemic core into the penumbra, ultimately leading to larger infarct volumes (Cavalcante et al., 2011; Muniyappa & Sowers, 2013; Stenmark et al., 2013). Thus, vascular dysfunction might likely serve as a mediator of aggravated stroke severity in stroke.

In addition to vascular mechanisms, inflammation represents an important, shared pathway linking obesity and stroke (Savopoulos et al., 2011). Obesity is characterized by chronic low-grade peripheral inflammation (Festa et al., 2001), which may prime the brain for an exaggerated inflammatory response either prior to or at onset of stroke. Following stroke, bidirectional communication between brain and the peripheral immune system further amplifies this response, making systemic inflammation an important contributor to disease progression and recovery (Kim & Cho, 2016). Importantly, fat distribution appears to be a critical determinant of cardiovascular risk. Visceral fat is more strongly associated with adverse outcomes than total body fat mass (The Emerging Risk Factors Collaboration, 2011). This may be explained by the endocrine function of adipose tissue, which actively secretes a variety of bioactive molecules, including adiponectin, leptin, resistin and cytokines, all of which are implicated in the regulation of vascular function and inflammation (Kawai et al., 2021; Ren et al., 2022). In this context, BMI has limitations as it does not consider body fat distribution or distinguish between lean and fat mass. Measures such as waist circumference or waist-to-hip ratio may therefore provide more accurate indicators of cardiovascular and metabolic risk (Pischoon et al., 2008).

Furthermore, obesity plays a central role in the development of metabolic syndrome, characterized by central obesity, impaired glucose metabolism, hypertension, and dyslipidemia. Within this context, obesity plays a particular role through its contribution to insulin resistance, a hallmark of T2D. However, not all individuals with obesity develop insulin resistance, and the mechanisms, underlying this variability are not fully understood. Emerging evidence highlights the importance of adipose tissue dysfunction in this process (Barazzoni et al., 2018; Guilherme et al., 2008; Qatanani & Lazar, 2007). Notably, approximately 30% of stroke patients have T2D (Fadini & Cosentino, 2018), underscoring the clinical relevance of

metabolic comorbidities in stroke risk and outcome. Consequently, obesity and metabolic syndrome are strongly associated with an increased risk of cardiovascular disease.

Sphingosine-1-Phosphate in Physiology and Disease

Sphingosine-1-phosphate (S1P) is a bioactive lipid that regulates several physiological and pathological processes. S1P is generated during sphingolipid metabolism, either from *de novo*-synthesized ceramide or by the degradation of sphingomyelin and glycosphingolipids at the plasma membrane or within lysosomes, thereby producing sphingosine, which is subsequently phosphorylated to form S1P (**Fig. 3**). Phosphorylation is catalyzed by two isoforms of sphingosine kinase (SphK), SphK1 and SphK2. SphK1 is primarily localized in the cytoplasm, whereas SphK2 is found both in the cytoplasm and predominantly in the nucleus (Proia & Hla, 2015). Although these isoforms exhibit distinct subcellular localization and functional roles, mice with a single *Sphk* knockout were viable, suggesting a compensatory mechanism between them (Allende et al., 2004; Mizugishi et al., 2005). Once formed, S1P can be exported by specific transporters, including spinster homolog 2 (SPNS2). S1P acts by binding to 5 G-coupled receptors (S1PR1-5), thereby mediating diverse signaling pathways (**Fig. 3**) (Proia & Hla, 2015). In contrast to the ubiquitous expression of S1PR1-3, S1PR4 and S1PR5 display a more tissue-specific distribution in the immune and nervous system. S1PR4 is primarily expressed in hematopoietic, lymphoid tissue and on immune cells and S1PR5 is predominantly found in the immune (T cells, natural killer cells) and nervous (oligodendrocytes) system (Gräler et al., 1998; Im et al., 2000; Jozefczuk et al., 2020). S1P levels are tightly regulated through balanced synthesis and degradation. S1P can be degraded by S1P lyase or dephosphorylated to sphingosine by S1P phosphatases (**Fig. 3**) (Proia & Hla, 2015). Thus, the coordinated activity of SphK, S1P lyase and phosphatase ensures precise control of cellular S1P concentrations (Venkataraman et al., 2008). In the circulation, S1P forms a characteristic vascular gradient. In plasma, it is primarily bound to carrier proteins such as albumin and high-density lipoprotein (HDL), whereas its levels in tissues are generally much lower (Kimura et al., 2001; Pappu et al., 2007; Schwab et al., 2005; Zhang et al., 2005). The main sources of plasma S1P include red blood cells (RBC), hematopoietic cells, and endothelial cells, which collectively contribute to maintaining this gradient and regulating S1P signaling (Hänel et al., 2007; Pappu et al., 2007; Venkataraman et al., 2008). Disruption of the vascular S1P gradient has been associated with vascular leakage, exaggerated inflammatory responses, defective lymphocyte egress, and abnormalities in vascular homeostasis and remodeling (Camerer et al., 2009; Hla et al., 2008; Spiegel & Milstien, 2011; Yanagida & Hla, 2017).

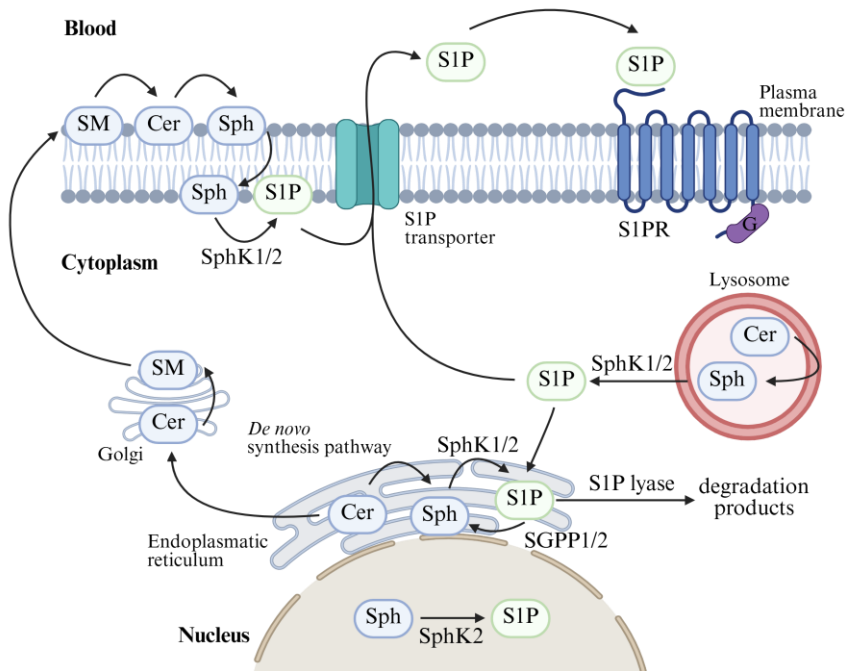


Figure 3| Sphingosine-1-phosphate metabolism and signaling.

Schematic overview of the sphingosine-1-phosphate (S1P) metabolism. S1P is generated from sphingosine (Sph), either from *de novo*-synthesized ceramide (Cer) or from the degradation of sphingomyelin (SM) and glycosphingolipids at the plasma membrane, within lysosomes, or in the endoplasmic reticulum. Sph is phosphorylated by the sphingosine kinase (SphK) isoforms SphK1 and SphK2 to form S1P. Cer can also be transported to the Golgi apparatus, where it is converted into SM before transported to the plasma membrane. Intracellular S1P can be exported by specific transporters, including spinster homolog 2 (Spns2), and acts via binding to five G protein-coupled S1P receptors (S1PR). Cellular S1P levels are tightly regulated by degradation through S1P lyase or dephosphorylation by S1P phosphatases (SGPP1/2) in the ER. Created with Biorender.com.

S1P Signaling in Ischemic Stroke

Growing evidence highlights the importance of S1P signaling in cardiovascular disease, including stroke, due to its role in vascular function and immune cell regulation. It contributes to multiple cellular mechanisms implicated in stroke pathology, including leukocyte chemotaxis, immune cell polarization, cytokine release, vascular function, and the maintenance of the endothelial barrier (Zhang et al., 2021). Through signaling via five G protein-coupled receptors (S1PR1-5), S1P influences both immune and cerebrovascular functions, making it an attractive therapeutic target for ischemic stroke.

Evidence from experimental stroke models demonstrate that S1P signaling is implicated in the response to cerebral ischemia. Upregulation of SphK1, one of the

S1P producing enzymes, S1P receptors, as well as increased brain S1P levels, have been observed after stroke, suggesting activation of this pathway during ischemic stroke (Kimura et al., 2008; Moon et al., 2015; Salas-Perdomo et al., 2019; Sun et al., 2017). Furthermore, treatment with fingolimod (FTY720), a functional S1PR modulator, attenuated S1P-mediated responses after transient middle cerebral artery occlusion (tMCAo), indicating that receptor-mediated S1P signaling contributes to ischemic brain damage (Moon et al., 2015).

Neuroinflammation is one of the key contributors to secondary brain injury, and studies demonstrating reduced infarct volumes in *Rag1*^{-/-} mice lacking T and B lymphocytes highlighted the importance of adaptive immune cells in stroke pathogenesis (Yilmaz et al., 2006). These findings increased interest in immunomodulatory strategies targeting lymphocyte trafficking. Fingolimod, an FDA- and EMA-approved treatment for relapsing-remitting multiple sclerosis, induces lymphocyte sequestration in lymph nodes primarily through functional antagonism of S1PR1 (Chun & Hartung, 2010; Cohen et al., 2010; Kappos et al., 2010). In several experimental stroke models, fingolimod reduced infarct size, BBB disruption and improved neurological outcomes (Wang et al., 2020; Wei et al., 2011; Xing et al., 2024). However, not all studies have reported beneficial effects in experimental models (Boisserand et al., 2025; Diaz Diaz et al., 2022; Salas-Perdomo et al., 2019). Interestingly, fingolimod's anti-inflammatory actions may not solely depend on S1PR1, as silencing of S1PR3 diminished its protective effects (Dong et al., 2018).

S1P signaling has also been described in the activation of resident glial cells. Microglia rapidly respond to ischemic injury and are major contributors to post-stroke neuroinflammation. Elevated SphK1 expression and enhanced S1P production have been detected in activated microglia following cerebral ischemia (Kimura et al., 2008; Zheng et al., 2015). Several S1P receptors, including S1PR1, S1PR2, and S1PR3, have been shown to regulate microglial activation and proliferation after stroke (Gaire et al., 2019; Gaire, Song, et al., 2018; Sapkota et al., 2019). Consistent with a detrimental role, knockdown of S1PR1 reduced microglial activation and proliferation, while both genetic deletion and pharmacological inhibition of S1PR2 reduced infarct size and improved neurological outcomes (Gaire, Lee, et al., 2018; Kim et al., 2015; Sapkota et al., 2019). These findings suggest that altered S1P signaling promotes microglia-driven neuroinflammation during ischemic stroke.

Astrocytes also represent important cellular targets of S1P signaling. S1P has long been known to stimulate astrocyte activation (Sorensen et al., 2003), and growing evidence indicates a particularly important role for S1PR3 in reactive astrogliosis after stroke (Gaire, Song, et al., 2018). Deletion of S1PR3 attenuated S1P-induced inflammatory responses in astrocytes (Dusaban et al., 2017), while pharmacological inhibition of S1PR3 with CAY10444 administered at time of reperfusion reduced infarct volumes and improved neurological function following stroke (Fan et al.,

2022; Gaire, Song, et al., 2018). These beneficial effects were associated with reduced activation of both microglia and astrocytes, further supporting a pro-inflammatory role of S1PR3 signaling in the ischemic brain (Gaïre, Song, et al., 2018).

In addition to the detrimental effects observed in immune and glial cells, S1P signaling has been implicated in endothelial function in stroke. Experimental studies have demonstrated impaired endothelium-dependent vasodilation and the development of a pro-inflammatory phenotype after stroke (Hu et al., 2017; Ishikawa et al., 2003). Notably, activation of endothelial S1PR1 strengthens the endothelial barrier integrity and reduces vascular leakage (Camerer et al., 2009; Huwiler & Zangemeister-Wittke, 2018). Consistent with this protective role, endothelial-specific S1PR1 deficient mice showed impaired microvascular perfusion after stroke (Nitzsche et al., 2021). Furthermore, selective S1PR1 agonists reduced infarct size and improved CBF in experimental stroke models. The beneficial effects of selective S1PR1 agonism were observed when treatment was initiated at reperfusion or up to six hours after vessel occlusion and were dependent on endothelial S1PR1 signaling (Brait et al., 2016; Hasegawa et al., 2010; Nitzsche et al., 2021).

Current evidence identifies S1P signaling as a central regulator of both inflammatory and vascular responses in ischemic stroke. Although preclinical and early clinical studies support the therapeutic potential of modulating S1P signaling, the precise receptor- and cell type-specific mechanisms remain incompletely understood. A better understanding of these complex effects will be essential for the development of more selective and effective S1P-based therapies for ischemic stroke.

S1P Signaling in Obesity and Metabolic Syndrome

Ceramides are bioactive sphingolipids and among the most studied lipid mediators in the pathogenesis of metabolic disease, being generally regarded as detrimental mediators of metabolic health. The accumulation of ceramides in insulin-sensitive tissues, particularly skeletal muscle, have been associated with the development of insulin resistance and T2D (Broskey et al., 2018; Chaurasia & Summers, 2015). In obesity, increased availability of fatty acids promotes the generation of several bioactive lipid molecules, including ceramides, which serve as precursors for the synthesis of S1P (Green et al., 2021). While the role of ceramides in the pathogenesis of metabolic disease is well established, the contribution of S1P signaling to obesity and metabolic syndrome remains not fully understood. Increasing evidence suggests that altered S1P signaling is a characteristic feature of both human and rodent obesity. Elevated circulating and tissue S1P levels, together with changes in S1P receptor expression profiles, have been reported in obese individuals and experimental models, indicating an important role of S1P signaling

in metabolic regulation (Kowalski et al., 2013; Skoug et al., 2024). Genetic deletion of *Sphk2* in mice has been shown to attenuate weight gain during high-fat diet (HFD) feeding and protect against age-related obesity and insulin resistance, suggesting that reduced SphK2 may confer metabolic benefits (Ravichandran et al., 2019; Zhao & Lee, 2023). However, findings from pharmacological inhibition have been less consistent. Notably, low-dose treatment with the selective SphK2 inhibitor ABC294640 failed to improve glucose tolerance, insulin sensitivity, body fat composition, or hepatic lipid handling in HFD-fed mice (Skoug et al., 2025). These results are consistent with a study reporting no significant effects on body weight following administration at higher doses (Maines et al., 2025). The discrepancies between genetic and pharmacological approaches may reflect differences in the respective obesity model, treatment duration and timing, or the different genetic or pharmacological approaches of SphK2 inhibition. Together, the effects of obesity and metabolic syndrome on S1P signaling appear to be highly context- and tissue-dependent. Several studies suggested beneficial actions of S1P metabolism in peripheral tissue, where S1P counteracted the development of insulin resistance in muscle, adipose tissue, and liver (Bruce et al., 2012; Lee et al., 2015; Ma et al., 2007; Osawa et al., 2011). In contrast, S1P signaling has also been shown to contribute to insulin resistance, particularly in the liver and in adipose tissue (Fayyaz et al., 2014; Wang et al., 2014). These contradictory findings underscore the complex and tissue-specific role of S1P signaling in the pathogenesis of metabolic disease exerting both protective and harmful effects depending on the cellular source of S1P, the SphK involved in its production, the receptor subtype activated, and the tissue examined (Green et al., 2021). Further investigation is required to define tissue-specific actions of S1P and to determine whether modulation of S1P signaling may represent a therapeutic strategy for treating obesity-related metabolic disorders.

Aims of the Thesis

The overall aim of this thesis was to investigate novel aspects of S1P signaling in ischemic stroke and obesity, with particular focus on expression of cell-specific receptor distribution, endothelial activation markers, neuroinflammation, and the therapeutic potential of S1PR3 antagonism as a post-stroke treatment in metabolically healthy and obese mice.

The specific aims of the papers included in the thesis were:

Aim I:

To determine whether ischemic stroke is associated with systemic S1P depletion accompanied by alterations in endothelial S1P pathway expression and elevation of endothelial activation markers.

Aim II:

To characterize the spatiotemporal expression profile of S1PR3 after ischemic stroke and evaluate the therapeutic potential of S1PR3 antagonism.

Aim III:

To investigate whether pharmacological SphK2 inhibition modulates obesity-associated neuroinflammation and microglial morphology.

Aim IV:

To assess how DIO shapes post-stroke S1P signaling and responsiveness to S1PR3-targeted therapy.

Key Methods

This section summarizes the key methods used in this thesis. For more detailed descriptions of the methods, please refer to the Material and Methods sections of the respective papers (see Appendix).

Animals and Surgical Procedures

All animal procedures were approved by the institutional ethics committee Malmö/Lund (Dnr. 5.8.18 – 01860/2021 or 5.8.18-05123/2021) or by LANUV of North Rhine-Westphalia (AZ #81-02.04.2019.A214) and conducted in accordance with the European animal protection laws (EU Directive 2010/63/EU), and reported following the ARRIVE guidelines.

For all mouse studies, male C57BL/6 mice were used, however they differed in their substrain depending on the disease model. For the stroke studies in **Papers I, II, and IV**, C57BL/6N were used, whereas C57BL/6J mice were used in the diet study presented in **Paper III**. The C57BL/6J mice is commonly used in metabolic and diet-induced studies (Fisher-Wellman et al., 2016), whereas strain differences have been less discussed in stroke studies (Zhao et al., 2019). Importantly, the C57BL/6J substrain carries a mutation in the nicotinamide nucleotide transhydrogenase (Nnt) gene, which has been shown to influence metabolism and immune responses (Freeman et al., 2006; Ripoll et al., 2012; Shimomura et al., 2009). In addition, both substrains differ in behavioral and physiological traits, including fear responses and cardiac responses to anesthetics (Radulovic et al., 1998; Roth et al., 2002; Stiedl et al., 1999). For the combined diet and stroke studies, we therefore chose the C57BL/6N substrain to enable comparison with previous stroke experiments from the lab (Battistella et al., 2021), while also characterizing its response to dietary intervention to ensure comparable metabolic phenotype development. Given the well-established sex discrepancies in experimental murine ischemic stroke models in susceptibility and outcome only male mice were used in all animal experiments to reduce biological variability and ensure a consistent experimental phenotype across studies. This discrepancy has been largely attributed to neuroprotective effects of estrogen in female mice, which results in smaller and a higher variability in infarct volumes depending on the estrous cycle (Jiang et al., 2020). Future studies should investigate whether the effects are also present in female mice, with careful

consideration of the estrous cycle and its potential impact on the time of stroke induction.

Preclinical Ischemic Stroke Mouse Model

Clinically, most ischemic strokes occur in the area of the middle cerebral artery (MCA), therefore, commonly used animal models resemble a blockage of the MCA to reproduce focal cerebral ischemia in rodents (Carmichael, 2005; Chiang et al., 2011; Sharma & Wong, 2022; Zeng et al., 2023). The MCA occlusion (MCAo) model offers several advantages, including high reproducibility, the presence of a penumbra, controllable reperfusion and the lack of requirement for craniectomy in the transient model. At the same time, limitations include increased hemorrhage risk, particularly in reperfusion models, and reduced suitability for studying thrombolytic therapies (Fluri et al., 2015). In tMCAo intraluminal occlusion of the MCA is followed by reperfusion through filament removal (**Fig. 4A**). This model reproduces ischemic injury, mimicking a clinical scenario in which blood flow is restored after spontaneous recanalization or therapeutic intervention, such as thrombectomy (Fluri et al., 2015). tMCAo was used in **Paper I, II** with the aim of investigating acute alterations in S1P signaling, endothelial dysfunction, and spatiotemporal S1PR3 expression under conditions involving reperfusion-associated injury. In contrast, permanent MCAo (pMCAo) induces sustained ischemia without reperfusion by electrocoagulation of the MCA (**Fig. 4B**). This model generates a stable ischemic lesion with limited variability in reperfusion-associated injury and reduced risk of secondary hemorrhage (Llovera et al., 2014). Typical lesion size and localization differ between permanent and transient MCAo models. pMCAo typically produces infarcts with substantial cortical involvement (**Fig. 4B**), whereas lesion characteristics in transient MCAo depend strongly on the duration of filament occlusion. Shorter occlusion periods result in primarily striatal infarcts, while longer occlusion periods lead to larger lesions involving both the striatum and cortical regions (**Fig. 4A**) (Bacigaluppi et al., 2010; Thaysen et al., 2024). As ischemic damage progresses gradually over time, pMCAo is particularly suitable for evaluating therapeutic interventions and post-stroke lesion expansion. Therefore, the pMCAo model was used in **Papers I, II, and IV** for therapeutic interventions investigating S1PR3 antagonism after stroke. In these studies, the pMCAo model allowed assessment of treatment efficacy during progressive ischemic injury and allowed evaluation of neurovascular and inflammatory responses independent of reperfusion-induced variability.

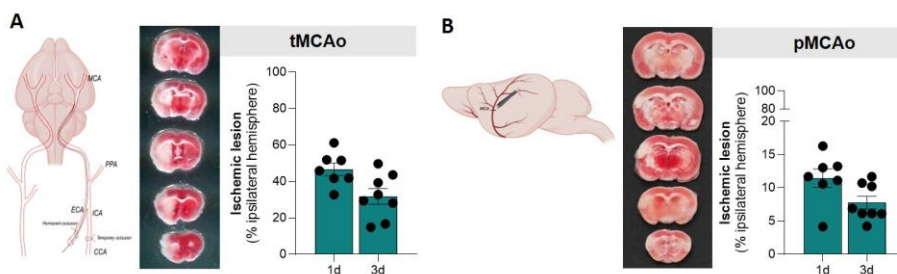


Figure 4| Experimental mouse models of ischemic stroke.

Schematic overview of the experimental stroke mouse models used and infarct size assessment. **(A)** Transient middle cerebral artery occlusion (tMCAo) and **(B)** permanent middle cerebral artery occlusion (pMCAo). Representative 2,3,5-triphenyltetrazolium chloride (TTC) staining is shown to visualize ischemic lesions, quantified as percentage of the ipsilateral hemisphere. *From Porschen et al., 2026.*

Sham-operated control mice were included in **Paper I, II, IV** to control the effects of anesthesia, surgical manipulation, tissue exposure, and perioperative inflammatory responses. Sham surgeries were performed using the same surgical procedures except for either monofilament insertion (tMCAo) or coagulation of the MCA (pMCAo).

Diet-induced Obesity

HFD-feeding was used to establish a model of DIO, characterized by increased body weight, fat content, hyperglycemia, thereby reflecting important metabolic aspects of human obesity. Plasma insulin levels were assessed using a commercially available ELISA kit (#10-1247-01, Mercodia), while whole-body fat and lean mass were determined by dual-energy X-ray absorptiometry (DEXA) in **Paper IV**. In **Paper III**, metabolic assessments were previously published elsewhere (Skoug et al., 2025). The DIO model was used for **Paper III, IV**, which aimed to investigate microglial morphology after SphK2 inhibition in DIO, and how obesity and altered metabolic status influence post-stroke S1P signaling dynamics.

Specifically, after one week of acclimatization, seven-week-old male C57BL/6N mice were started on dietary intervention. Mice were fed a control diet (CD) containing 10% kcal from fat, 20% kcal from protein, and 70% kcal from carbohydrates for one week, followed by either CD or HFD with 60% kcal from fat, 20% kcal from protein and 20% kcal from carbohydrates for 8 consecutive weeks. Both diets were open-source purified diets obtained from Research Diets (CD: D12450J, HFD: D12492). The inclusion of a nutrition-matched CD was important because purified diets differ from rodent chow diet (RCD) in nutrient composition, fiber content, fat source, and micronutrient composition (González-Blázquez et al., 2020; Kang et al., 2013; von Süßkind-Schwendi et al., 2023; Warden & Fisler,

2008). These factors may influence metabolism, inflammation, gut microbiota composition, as well as S1P signaling pathways (Maceyka & Spiegel, 2014; Norris & Blesso, 2017). The CD group therefore allowed for discrimination between effects caused by obesity itself and those related to diet composition. This was specifically of relevance in **Paper IV**, where both CD and HFD exposure altered S1P signaling responses compared with RCD-fed mice.

In **Paper I-II and IV**, mice were fed a standard RCD (SAFE A30, SAFE Diets, Augy, France) provided by the animal facility. Under these conditions, RCD provided a baseline model without introducing additional metabolic confounders.

Human Cohort

All human investigations were performed in accordance with the principles outlined in the Declaration of Helsinki. Plasma samples included in **Paper I, II** were selected from the Lund Stroke Recovery Study (Aked et al., 2020), a sub-cohort of the Lund Stroke Register comprising individuals with first-ever stroke events from eight municipalities in southern Sweden. The study was approved by the Regional Ethical Review Board in Lund, Sweden (Registration No. 2016/179) and the Swedish Ethics Review Board (Registration No. 2020-07047). Participants gave informed consent prior to inclusion to the study. Fifty patients, based on *a priori* sample size calculation, were randomly selected and age- and sex-matched with control subjects. Baseline characteristics were obtained from the regional Biobank (Biobank Sverige, Södra Sjukvårdsregionen). A limitation of the cohort is that the included patients generally presented with mild stroke symptoms, with a median National Institutes of Health Stroke Scale (NIHSS) score of 2.5 (IQR: 1.0 – 4.2). Therefore, the findings may not be fully generalizable to patients with moderate or severe ischemic stroke. Although the sample size was determined by *a priori* sample size calculation, the relatively limited number of patients may have reduced the ability to detect smaller effect sizes and limited subgroup analyses.

Preparation and Administration of S1P-targeted Therapies

S1PR3 antagonist CAY10444 (#10005033, Cayman Chemicals) was dissolved in 0.375% Tween-80 in saline or PBS and the pH was adjusted to 7.5. One mg/kg CAY10444 or vehicle (saline or PBS) were injected intraperitoneally 4-, 6- and 8-hours in **Paper II** or 4-hours or 4-hours and 24-hours in **Paper IV** after pMCAo.

In **Paper III**, mice received 5 mg/kg of SphK2 inhibitor ABC294640 (BioNordika) or vehicle (0.3%(v/v) ethanol in 50:50 polyethylene glycol (PEG)/H₂O) after 7 weeks of diet (CD or HFD) subcutaneously every second day for two weeks.

Separation of Brain Tissue into Vessel-rich and Vessel-depleted Fractions

Brain tissue fraction was performed from individual brain hemispheres (ipsilateral and contralateral hemisphere) as previously described (Matthes, 2021). Briefly, hemispheres were homogenized in HBSS (containing 10 mM HEPES) and centrifuged to separate the vessel-depleted parenchymal fraction from the vessel-containing pellet. Vessel-rich fractions were subsequently isolated using dextran density (HBSS containing 10 mM HEPES and 18% dextran ~ 70000) centrifugation, bovine serum albumin (BSA; 1%) washing steps, and filtration through a 20 µm cell strainer (43-10020, Pluriselect). Isolated vessel fractions were either subsequently used for staining (**Paper I**), or lysed in RIPA buffer containing protease and phosphatase inhibitors and homogenized prior to protein analyses, and Western blotting (**Paper I and II**).

Brain Tissue Preparation for Molecular Analysis

Mice were transcardially perfused with 0.9% saline at 1-, 3- or 7-days post-stroke, or at the end of the dietary intervention. In **Paper I, II and IV**, brains were extracted and separated into ipsilateral (ischemic) and contralateral hemispheres (control). In **Paper III** both hemispheres were used for homogenization. In the diet comparison cohort of **Paper IV** without stroke intervention, one hemisphere was used for homogenization, whereas the other one was used for immunohistochemistry. In **Paper III**, the hemisphere was further dissected into cortex and hippocampus, which were homogenized separately. The respective hemisphere or brain fraction was homogenized in PBS and stored at -80°C until further processing. This approach enabled tissue processing for multiple downstream analyses, such as RNA and protein isolation or S1P extraction.

RNA Preparation and RT-qPCR

Whole hemisphere or brain fraction homogenates were dissolved in TRIzol (#15596018, Thermo Fisher) and processed according to manufacturer's instructions for total RNA isolation. Subsequently, 500 ng or 1 µg of RNA was reverse transcribed to cDNA using the High-Capacity cDNA Reverse Transcriptase

Kit (#4368814, Thermo Fisher). Gene expression was detected using SYBR Green PCR Master Mix (#4369702, Thermo Fisher) on a C1000 Touch Thermal Cycler (Bio-Rad). All samples were run in triplicates, and relative gene expression was calculated using a standard curve-based approach and normalized to the corresponding housekeeping gene *Rpl14* or *GPI*. qPCR data are presented as values of their corresponding group or normalized to the relevant control condition (normoxia in **Paper I** or contralateral hemisphere in **Paper II, IV**). A list of primers used is provided in **Table 1, 2**.

Western Blot

Western blot was performed on isolated cerebral vessel or parenchyma fraction (**Paper I, II**), whole brain tissue homogenates (**Paper I**), or cortical and hippocampal fractions (**Paper III**). Briefly, samples were lysed in a RIPA-based buffer containing phosphatase and protease inhibitors, and insoluble material was removed by centrifugation. Protein concentrations were determined using Pierce BCA Protein Assay Kit (23227, Thermo Fisher), followed by separation in SDS-PAGE mini gels and transfer on PVDF membranes. Membranes were blocked for 1 h at room temperature in 5% non-fat dry milk (in PBS containing 0.05% Tween-20), incubated with primary antibodies overnight at 4 °C, followed by HRP-conjugated secondary or Alexa Fluor-conjugated secondary antibody incubation for 1 h at room temperature. Proteins from HRP-conjugated secondary antibody incubation were visualized by enhanced chemiluminescence. Relative protein expression was analyzed by densitometry and normalized to housekeeping proteins or total lane protein. A list of antibodies used is provided in **Table 3**.

S1P Quantitation by Mass Spectrometry

S1P levels were quantified in human (**Paper I**) or murine plasma (**Paper I, IV**), brain fractions including cortex and hippocampus (**Paper III**), or whole brain hemispheres (**Paper IV**) using liquid chromatography-coupled tandem mass spectrometry on a 6495 QQQ instrument (Agilent Technologies, Sweden). Briefly, brain tissue homogenates equivalent to 10 mg were processed by lipid extraction using a methanol/chloroform-based protocol, whereas plasma samples were extracted using ice-cold methanol. In all experiments, the internal standard S1P-D7 (860659P, Avanti Polar Lipids, Merck) was added prior to extraction to ensure accurate quantification.

Immunohistochemistry

Immunohistochemistry was performed on 10 µm brain sections (**Paper II**), 30 µm free-floating brain sections (**Paper III**), or purified cerebral vessels (**Paper I**). For

vessel stainings, purified vessels were mounted on poly-D-lysine-coated cell culture slides (#94.6170.402, Sarstedt), fixed in 4% paraformaldehyde (PFA) for 30 min, and permeabilized with 0.2% Triton X-100 in PBS for 15 min, followed by PBS washes. For brain tissue stainings, mice were transcardially perfused with 0.9% saline prior to brain removal. Brains were fixed in 4% PFA for 24 h at 4 °C, cryoprotected in a 15-30% sucrose gradient in PBS, and stored at 4 °C until sectioning. Samples were incubated in blocking buffer (11096176001, Roche) for 30 min at room temperature, followed by overnight incubation with primary antibodies at 4 °C. For CD31 staining in **Paper II**, antigen retrieval was performed prior to blocking by incubation in 1% SDS in PBS for 7 min at room temperature, followed by PBS washes. After primary antibody incubation, samples were washed in PBS and incubated with Alexa Fluor-conjugated secondary antibodies for 1 h at room temperature. Following PBS washes, sections were mounted using Fluoromount-G Mounting Medium (00-4958-02, Thermo Fisher) or Fluoromount-G Mounting Medium with DAPI (00-4959-52, Thermo Fisher). A list of antibodies used is provided in **Table 3**.

Infarct Size Determination

Infarct size following MCAo was determined either by 2,3,5-Triphenyltetrazolium chloride (TTC) staining (**Paper I, II**) or by T2-weighted magnetic resonance imaging (MRI; **Paper II, IV**).

TTC Staining

Following transcardiac perfusion, brains were sectioned into 1 mm coronal slices using a brain matrix and incubated in 2% TTC (93140, Sigma-Aldrich) dissolved in 0.9% saline for 15-30 min at 37 °C. Brain sections were subsequently imaged using a microscope, and infarct lesion size was analyzed using ImageJ. Lesion size was presented as percentage of ipsilateral (**Paper I**) or contralateral hemisphere (**Paper II**). For tMCAo, infarct size was presented as percentage of the contralateral hemisphere to account for ischemia-associated swelling of the ipsilateral hemisphere. However, recent studies suggest that edema correction may require more advanced normalization approaches to more accurately estimate infarct volume under conditions of substantial tissue swelling (Nouraei et al., 2019).

MRI

In **Paper II, IV**, MRI was performed using a preclinical 9.4 T scanner (Bruker) equipped with a quadrature volume resonator coil (112/087) for transmission and a

mouse brain 2x2 phased array coil for reception. Mice were anesthetized using isoflurane, while body temperature was maintained at 36-37 °C and respiration rate at 70-100, which were continuously monitored. Whole-brain T2-weighted images were acquired using a Rapid Imaging with Refocused Echoes (RARE) sequence. Infarct volume was analyzed using ImageJ and either presented as percentage of ipsilateral or contralateral hemisphere.

Statistical Analyses

Statistical analysis was performed using GraphPad Prism 9-11 software, whereas MATLAB was used for selected analyses in **Paper III**. Normality was tested using Shapiro-Wilk test or by Q-Q plot inspection. Normally distributed data was presented as mean $\pm$ SEM and analyzed using parametric tests, whereas non-normally distributed data was presented as median $\pm$ interquartile range (IQR) and analyzed using non-parametric tests. Comparisons between two independent groups were analyzed using Student's t-test or Mann-Whitney test. Comparisons between multiple independent groups were analyzed using one-way analysis of variance (ANOVA) followed with appropriate post-hoc testing, including Tukey's, Sidak's or Fisher's least significant difference (LSD) post-hoc test, or analyzed by Kruskal Wallis test with Dunn's post-hoc test. Repeated measurements were analyzed using repeated-measures two-way ANOVA followed with appropriate post-hoc testing, including Tukey's, Sidak's or Fisher's LSD post-hoc test. Correlation analyses were performed using either Pearson or Spearman correlation analysis depending on data distribution. In **Paper III**, non-parametric approaches due to small sample size were used, including aligned rank transform (ART) ANOVA for grouped (factorial) analyses. Statistical significance was defined as $p < 0.05$.

Table 1| Primers used for RT-qPCR in mouse.

Target	Forward primer (5'→ 3')	Reverse primer (5'→ 3')	Paper
<i>Rpl14</i>	GGCTTTAGTGGATGGACCCT	ATTGATATCCGCTTCTCCC	I - IV
<i>Sphk1</i>	GGTGAATGGGCTAATGGAACG	CTGCTCGTACCCAGCATAGTG	I, III, IV
<i>Sphk2</i>	CACGGCGAGTTTGTTCCCTA	CTTCTGGCTTTGGGCGTAGT	I, III, IV
<i>Sgpp1</i>	GAGCAACTTGCCGCTCTACTA	GGTCGAGATTCCAGATCCAGAA	I, III
<i>Sgpp2</i>	GTTCTCTACGCTGGTGTGTCT	GCAGGGTAGGTCAGAGCAAT	I, III
<i>Sgpl1</i>	GGTGTATGAGCTTATCTTCAGC	CTGTTGTTCGATCTTACGTCCA	I, III
<i>S1pr1</i>	ATGGTGTCCACTAGCATCCC	CGATGTTCAACTTGCCTGTGTAG	I, III, IV
<i>S1pr2</i>	ACAGTCTCCAAAACCAACCACT	ACTCTGAGTATAAGCCGCCCA	I, III, IV
<i>S1pr3</i>	CAAGCCTAGCGGGAGAGAAA	ACTGCGGGAAGAGTGTTGAA	I - IV
<i>S1pr4</i>	CTCGGTCACTCAGCTTCAGG	CAGTCACCATCAGGCAAGGT	I, IV
<i>S1pr5</i>	GCCTAGGGCACACGACC	GACACAGGAGAGCGGCTATT	I, IV
<i>Selp</i>	CATCTGGTTCAGTGCTTTGATCT	ACCCGTGAGTTATTCCATGAGT	I
<i>Sele</i>	CACGACATGCACGTTTGACT	GGCGCAGATAAGGCTTCACA	I
<i>Icam1</i>	CCGTGCCAAGCCACGCTAC	GCGGAAGCGGACGACTGCAC	I
<i>Vcam1</i>	TGCCGAGCTAAATTACACATTG	CCTTGTGGAGGGATGTACAGA	I, IV
<i>Pecam1</i>	CCAAAGCCAGTAGCATCATGGTC	GGATGGTGAAGTTGGCTACAGGG	I
<i>Cdh5</i>	ATGGCAGGCCCTAACTTTCC	CATTCTGGCGGTTACGTTG	I
<i>Vegfa</i>	GGCCTCCGAAACCATGAACT	GTCCACCAGGGTCTCAATCG	I
<i>Adm</i>	TTCTCGGCTTCTCATCGCAG	TCAGGGTGATGGAACCAGC	I
<i>Hmox1</i>	GAATCGAGCAGAACCAGCCT	CTCAGCATTCTCGGCTTGGA	I
<i>Ocln</i>	GAAAGGGCAAGAGGACGGAC	CGTCGTCTAGTTCTGCCTGT	I
<i>Gfap</i>	AAGGTCCGCTTCCTGGAA	GGCTCGAAGCTGGTTCAGTT	II, IV
<i>Glt-1</i>	CCGACCGTATAAAATGAGCTACC	ATTCCTGTGACGAGACTGGAG	II, IV
<i>Gbp2</i>	TGCTGGATCTTTGCTTTGGC	TTAGCGGAATCGTCTACCCC	II, IV
<i>Emp1</i>	CTGTTTGTCTCCACCATTGCC	ACCACCAGTGCAGTTCTTCC	II, IV
<i>Il6</i>	TCTGAAGGACTCTGGCTTTG	GATGGATGCTACCAAACCTGGA	III
<i>Tnfa</i>	TTGACCTCAGCGCTGAGTTG	CCTGTAGCCACGTCGTAGC	III
<i>Il1b</i>	GAAGAGCCCATCCTCTGTGA	TTCATCTCGGAGCCTGTAGTG	III
<i>Cd68</i>	TGTCTGATCTTGCTAGGACCG	GAGAGTAACGGCCTTTTGTGA	III, IV
<i>Nos2</i>	GGCAGCCTGTGAGACCTTTG	GCATTGGAAGTGAAGCGTTTC	III
<i>Mcp-1</i>	AGGTGTCCCAAAGAAGCTGTAG	AATGTATGTCTGGACCCATTCC	IV

Table 2| Primers used for RT-qPCR in human.

Target	Forward primer (5'→ 3')	Reverse primer (5'→ 3')	Paper
GPI	AGGCTGCTGCCACATAAGGT	CCAAGGCTCCAAGCATGAAT	
SPHK1	AGGCTGAAATCTCCTTCACGC	GTCTCCAGACATGACCACCAG	
SPHK2	CTGTCTGCTCCGAGGACTGC	CAAAGGGATTGACCAATAGAAGC	
SGPP1	CACCGCCATCCCCATTCTA	AACTAGAGAACACCAGCAGGG	
SGPP2	CCATTGCCTTCACCCTCCTT	CCAGTGTAGAGCCTGCTGAG	
SGPL1	ACTGCTCGCTTCCTCAAGTC	TACTGGGTGGGAAGTCAAC	
S1PR1	TCTGCGGGAAGGGAGTATGT	CGATGGCGAGGAGACTGAA	
S1PR2	CCTTCTCTGGGCAACTTCGT	GGAGAAGTCGTCAAGTGGCA	
S1PR3	TCTACCATCCTGCCCTCTAC	ACACGCTCACCACAATCACC	
S1PR4	CAACCCCATCATCTACTCCTTCC	AAAGCTGTCCCTTGGCCTC	
S1PR5	TGTATTTATGCAGCGACACCCC	CTTCCCTGACCACCATCACC	
VCAM1	CATCCGAAAGGCCAGTTGA	TAGAGCACGAGAAGCTCAGGA	
PECAM1	AAGTGGAGTCCAGCCGCATATC	ATGGAGCAGGACAGGTTCACTC	
CDH5	CGGCGCCAAAAGAGAGATTG	CGTCTCCTGTCTCTGCATCG	
VEGFA	AAGGAGGAGGGCAGAATCATC	TCAGGGTACTCCTGGAAGATGT	
ADM	CTTCGGAGTTTTGCCATTGCC	CTGGACATCCGCAGTCCCC	
HMOX1	AACTTTCAGAAGGGCCAGGT	CTCCTCCAGGGCCACATAGA	

Table 3| Primary antibodies used for western blot and immunofluorescence.

Antibody	Host	Company	#Catalogue number	Application/ Dilution	Paper
CD31	Goat	R&D Systems	AF3628	IHC: 1:200	I
GFAP	Rat	Thermo Fisher Scientific	13-0300	IHC: 1:200	I
SPHK1	Rabbit	abcam	ab71700	IHC: 1:200	I
SPHK2	Rabbit	Proteintech	17096-1-AP	IHC: 1:200	I, III
S1PR1	Rabbit	Thermo Fisher Scientific	PA1-1040	WB: 1:1000	I
S1PR4	Rabbit	Novus	NBP2-24500	WB: 1:1000	I
CD13	Rat	BioRad	MC2183	IHC: 1:300	II
CD31	Mouse	abcam	ab24590	IHC: 1:200	II
GFAP	Goat	abcam	ab53554	IHC: 1:500	II
Lectin	Tomato	Sigma	L0401	IHC: 1:200	II
Serum albumin	Goat	R&D Systems	AF3329	WB: 1:1000	I, II
VE-Cadherin	Rabbit	abcam	ab33168	WB: 1:1000	I
GFAP	Goat	Agilent	Z0334	WB: 1:1000	II
S1PR3	Rabbit	OriGene	TA329055	WB: 1:2000, IHC: 1:200	II
Iba1	Rabbit	FUJIFILM Wako	019-19741	IHC: 1:250	III
Iba1	Guinea pig	Synaptic Systems	234 308	IHC: 1:500	III
Phospho-S1PR1	Rabbit	Thermo Fisher Scientific	PA5-40299	WB: 1:1000	III

Summary of Results and Discussion

Systemic and Endothelial Alterations in S1P Signaling after Acute Ischemic Stroke (**Paper I**)

Plasma S1P levels were significantly reduced in patients with acute ischemic stroke compared with age- and sex-matched controls (**Fig. 5A**), providing validation of previous observations (Lee et al., 2022) in our study population. Consistently, a similar reduction was observed in the murine pMCAo model relative to sham-operated mice (**Fig. 5B**). Notably, in the pMCAo model, S1P levels remained within the range of naïve mice but were decreased compared with sham controls (1d Sham: 592.7 ± 126.4 ; 1d pMCAo: 489.1 ± 103.6 ; 3d Sham: 468.0 ± 65.0 ; 3d pMCAo: 440.0 ± 163.2 ; naïve: 482.5 ± 114.8 nmol/L), suggesting that procedure-related factors such as anesthesia, tissue trauma, stress responses, and inflammation may contribute to the stroke-related changes in circulating S1P levels. Given that reduced plasma S1P has also been described in conditions of endothelial dysfunction and vascular stress, such as preeclampsia (Edvinsson et al., 2025), we investigated whether systemic S1P alterations are accompanied by changes in endothelial activation and S1P signaling. We therefore analyzed endothelial mRNA expression of S1P signaling components using endothelial-specific RiboTag mice ($Cdh5^{Cre-ER(T)}/RiboTag$) after tMCAo. This revealed a significant reduction in endothelial transcript levels of S1P generating enzymes, including *Sphk2* in the ipsilateral compared to the contralateral hemisphere 1-day after stroke (**Fig. 5C**). This was supported by reduced endothelial SPHK2 protein intensity in isolated cerebral vessels (**Fig. 5D**). These findings indicate an acute suppression of the endothelial S1P-generating enzyme SphK2 following ischemic stroke. While SphK2 is known to contribute substantially to circulating S1P via erythrocytes, platelets, and other non-endothelial sources (Meissner et al., 2017; Sensken et al., 2010; Urtz et al., 2015), its downregulation in the cerebrovascular endothelium suggests a compartment-specific alteration in local S1P signaling that may contribute to post-stroke vascular dysfunction.

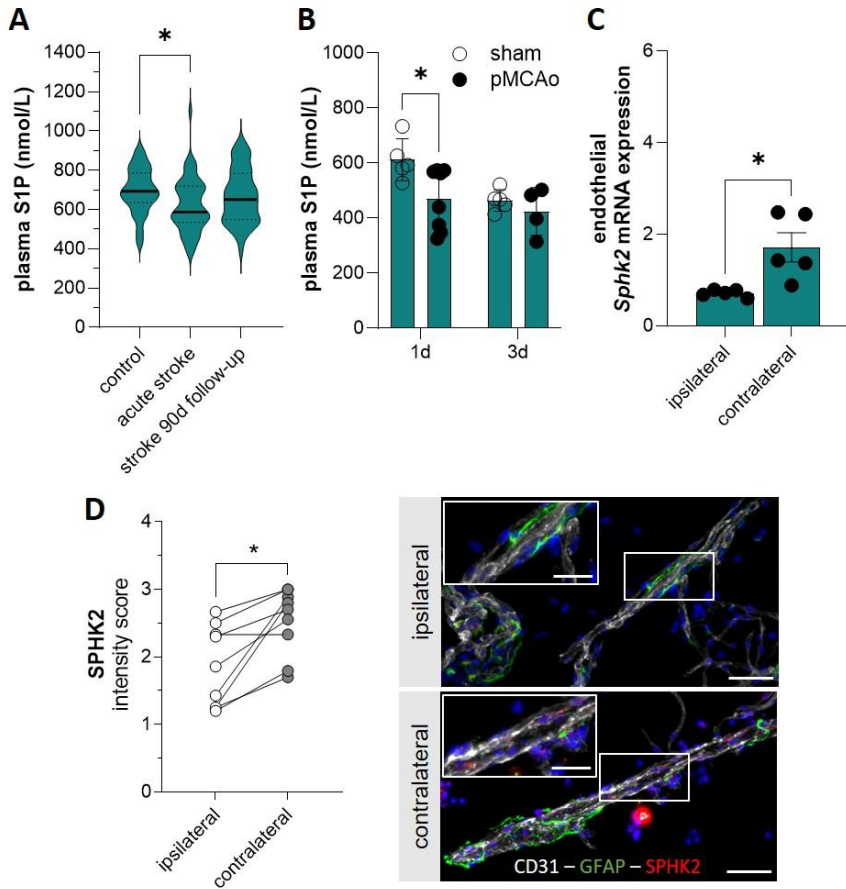


Figure 5| Reduced circulating S1P and endothelial SphK2 regulation after acute ischemic stroke. (A) Plasma sphingosine-1-phosphate (S1P) levels in healthy controls, patients with acute ischemic stroke, and at 90-days follow-up (controls N = 47; stroke N = 50). (B) Plasma S1P levels 1- and 3-days after permanent middle cerebral artery occlusion (pMCAo) in mice (sham N = 5; pMCAo N = 8). (C) Endothelial sphingosine kinase (*Sphk*)2 expression in ipsilateral and contralateral hemispheres 1-day after transient middle cerebral artery occlusion (tMCAo) in *Cdh5^{Cre-ER(T)}/RiboTag* mice (N = 5). (D) Representative immunofluorescence and quantification of endothelial SPHK2 intensity (score: 0 = no detectable signal, 1 = low, 2 = moderate, 3 = strong, 4 = very strong) in cerebral vessel isolated 1-day after pMCAo stained for 4',6-diamidino-2-phenylindole (DAPI), cluster of differentiation 31 (CD31), glial fibrillary acidic protein (GFAP) and SPHK2, comparing ipsilateral compared to contralateral vessels. Scale bars represent 30 μ M. Data are shown as median $\pm$ interquartile range and analyzed using the Mann-Whitney test (A) or as mean $\pm$ SEM (B – D) and analyzed by two-way ANOVA (B) or paired t-test (C, D). * P < 0.05. Adapted from Porschen et al., 2026.

The reduction in plasma S1P levels was accompanied by decreased endothelial *S1pr1* expression in ipsilateral compared to the contralateral hemisphere 1-day after stroke (**Fig. 6A**), suggestive of reduced endothelial S1PR1-mediated signaling within the ischemic cerebrovasculature. This was further supported by an inverse relationship between cerebrovascular S1PR1 protein expression and parenchymal serum albumin accumulation, indicative for BBB leakage (**Fig. 6B**). A key function of endothelial-derived S1P is to maintain vascular homeostasis, which signals predominantly through endothelial S1PR1 to promote endothelial junctions and barrier function (Anwar & Mehta, 2020; Christoffersen et al., 2011; Levesque et al., 2025; Levesque & Hla, 2023; Liu et al., 2000; Xiong & Hla, 2014). In line with impaired barrier function, occludin (*Ocln*) expression was reduced in the ipsilateral hemisphere (**Fig. 6C**), whereas P-selectin (*Selep*) was upregulated compared with the contralateral hemisphere at 1-day after stroke (**Fig. 6D**). Disruption of S1P signaling has previously been linked to endothelial activation (Nitzsche et al., 2021; Wilkerson et al., 2012; Zheng et al., 2025), including increased expression of adhesion molecules such as P-selectin (Matsushita et al., 2004; Nussbaum et al., 2015; Zheng et al., 2025).

Interestingly, an inverse relationship between plasma S1P and circulating P-selectin levels were observed in murine (**Fig. 6E**) and human stroke (Porschen et al., 2026), relating acute post-stroke plasma S1P reductions with elevation of endothelial activation and BBB dysfunction markers.

Importantly, these findings are primarily based on transcriptomic and correlative analyses and therefore provide descriptive evidence of S1P pathway dysregulation. They do not establish a direct causal link or define the endothelial-specific contribution to circulating S1P depletion after stroke, underscoring the need for further mechanistic studies.

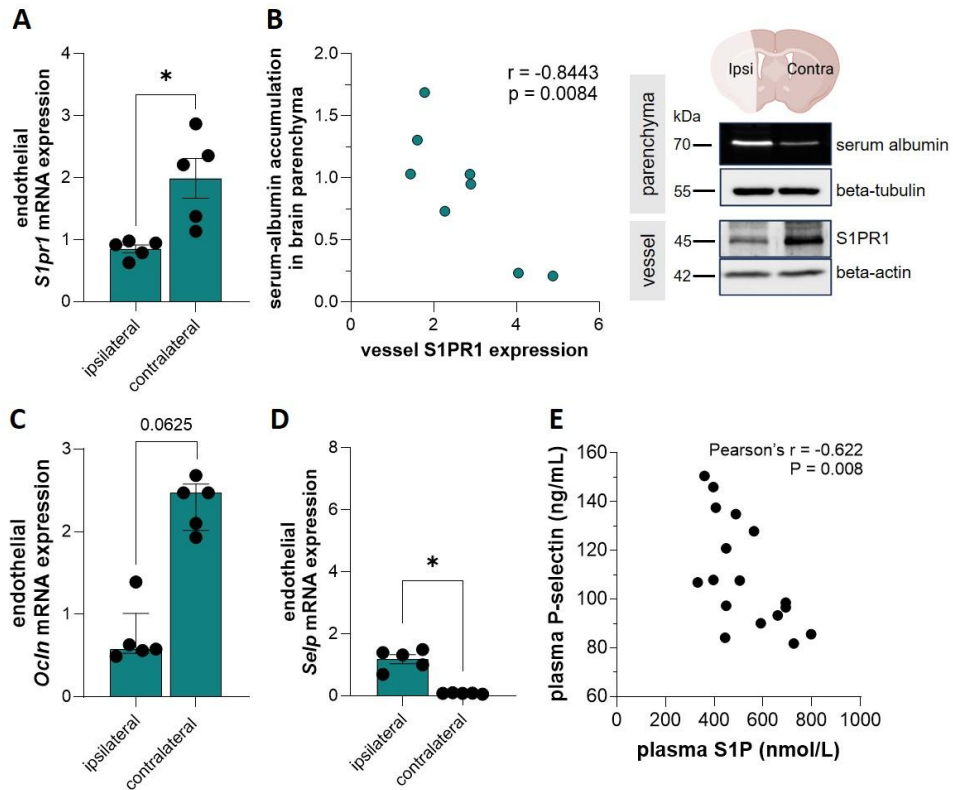


Figure 6| Endothelial S1P signaling and activation-related marker expression after ischemic stroke.

(A) Endothelial sphingosine-1-phosphate (*S1pr*)1 expression from ipsilateral and contralateral hemispheres 1-day after transient middle cerebral artery occlusion *Cdh5^{Cre-ER(T)}/RiboTag* mice (tMCAo; N = 5). (B) Correlation between vessel S1PR1 protein expression and parenchymal serum albumin accumulation after permanent middle cerebral artery occlusion (pMCAo), indicating blood-brain barrier leakage (N = 6; representative immunoblots are shown). (C, D) Endothelial mRNA expression of occludin (*Ocln*) (C) and P-selectin (*Selp*) (D) from ipsilateral and contralateral hemispheres after tMCAo *Cdh5^{Cre-ER(T)}/RiboTag* mice (N = 5). (E) Correlation between plasma P-selectin and sphingosine-1-phosphate (S1P) levels 1-day after tMCAo and sham surgery. Data are shown as mean $\pm$ SEM (A, D) and analyzed by paired t-test (A, D) or median $\pm$ interquartile range (C) and analyzed by Wilcoxon matched-pairs signed rank test (C) or Pearson correlation (B, E). *P < 0.05. Adapted from Porschen et al., 2026.

Ipsilateral S1PR3 Expression is Upregulated Post-stroke and S1PR3 Antagonism Treatment after Ischemic Stroke (Paper II)

Targeting S1P signaling has gained increasing attention in ischemic stroke therapy (Sun et al., 2016; Zhang et al., 2021; Zhang et al., 2022). However, a detailed understanding of temporal and cell-specific S1P signaling dynamics is essential for successful clinical translation. We therefore investigated cerebrovascular S1PR3 responses after ischemic stroke and the effects of genetic deletion or S1PR3 antagonism treatment on stroke outcome.

We observed a significant upregulation of *S1pr3* in the ischemic hemisphere at 1-day and 3-days after stroke (**Fig. 7A**), validating previous observation and extending them by showing a distinct spatiotemporal expression profile of S1PR3 (Matuskova et al., 2024; Salas-Perdomo et al., 2019). Consistently, genetic deletion of *S1pr3* resulted in reduced infarct size compared to wildtype controls (**Fig. 7B**), supporting an important role of S1PR3 signaling in the acute phase of ischemic stroke. To further define the cellular sources of this response, we examined S1PR3 expression across vessel-associated and parenchymal cell-types. Immunofluorescence and quantitative analyses revealed a predominantly vessel-associated localization of S1PR3. S1PR3 is highly expressed in astrocytes, with lower expression in endothelial cells and pericytes (**Fig. 7C**). This was further confirmed by astrocyte-specific transcriptome profiling in astrocyte-specific RiboTag (Cnx43^{Cre-ER(T)}/RiboTag) mice, showing an acute upregulation of astrocytic *S1pr3* in the ipsilateral hemisphere following tMCAo (**Fig. 7D**). Furthermore, S1PR3 antagonism using TY52165 attenuated *Gfap* expression compared to vehicle-treated mice (**Fig. 7E**), confirming an important role of S1PR3 in astrocyte activation (Zamanian et al., 2012). These findings reveal a distinct spatiotemporal expression profile of S1PR3 during the acute phase of ischemic stroke. Taken together, they support a model in which S1PR3 acts as a key modulator of early astrocyte-mediated responses to ischemic injury and represents a potential therapeutic target following ischemic stroke.

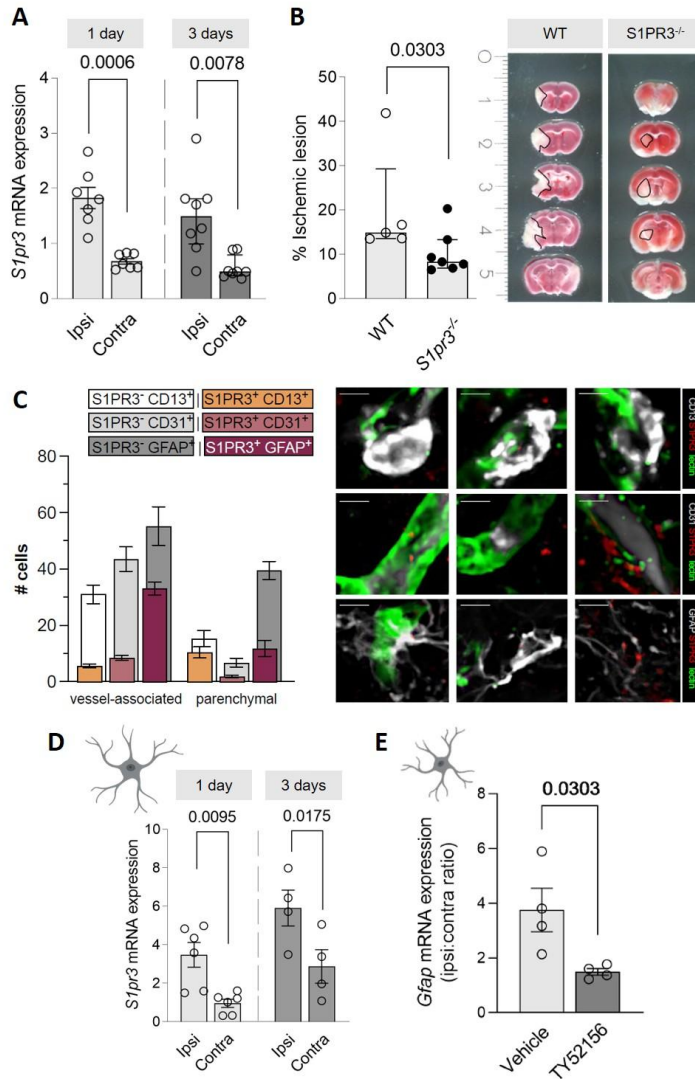


Figure 7| S1PR3 is upregulated after ischemic stroke and its cell-specific expression within the neurovascular unit.

(A) Sphingosine-1-phosphate receptor 3 (*S1pr3*) expression in ipsilateral (ipsi) and contralateral (contra) hemispheres 1-day and 3-days after transient middle cerebral artery occlusion (tMCAo; N = 7-8). (B) Infarct size 1-day after stroke in wildtype (WT) and *S1pr3* knockout (*S1pr3*^{-/-}) mice (N = 5-7; representative TTC staining shown, scale bar in cm). (C) Representative immunofluorescence and quantification of vessel-associated and parenchymal S1PR3 localization of CD13⁺, CD31⁺, and glial fibrillary acidic protein (GFAP)⁺ cells. (D) Astrocyte-specific *S1pr3* expression in ipsi and contra hemispheres 1-day and 3-days post-tMCAo in Cnx43^{Cre-ER(T)}/RiboTag mice (N = 4-6). (E) *Gfap* mRNA expression following S1PR3 antagonism (TY52156) compared to vehicle controls in Cnx43^{Cre-ER(T)}/RiboTag mice (N = 4). Data are mean ± SEM (A: 1-day, C - E) or median ± interquartile range (A: 3-days, B) and analyzed by paired t-test (A: 1-day), Student's t-test (D, E), Wilcoxon matched-pairs signed rank test (A: 3-days) or Mann-Whitney test (B). *P < 0.05. Adapted from Matuskova et al., 2024.

To evaluate the therapeutic potential of targeting S1PR3, we administered the S1PR3 antagonist CAY10444 at clinically relevant timepoints (4, 6 and 8 h) after stroke onset in the permanent MCAo model. While previous studies reported reduced lesion size when treatment was initiated at the time of reperfusion (Gaire, Song, et al., 2018), we wanted to assess efficacy within a later treatment window. Notably, infarct size was reduced when treatment was administered at 4 h post-stroke, whereas no effect on infarct size and brain perfusion were observed at later timepoints (**Fig. 8A**). This was accompanied by reduced BBB leakage, as reflected by reduced parenchymal serum albumin accumulation at the 4 h administration timepoint (**Fig. 8B**). Importantly, later administration of the S1PR3 antagonist did not negatively affect BBB integrity as assessed by parenchymal serum-albumin accumulation (**Fig. 8B**). The limited therapeutic window during which S1PR3 antagonism improves infarct size and cerebral perfusion may be related to the temporal dynamics of S1PR3 expression. Previous work has shown a progressive increase in *S1pr3* levels within hours after stroke, with marked upregulation at six hours (Salas-Perdomo et al., 2019). It is therefore possible that at later timepoints, S1PR3 expression exceeds a threshold at which pharmacological antagonism becomes less effective. However, our findings suggest that more selective targeting of individual S1P receptors may be preferable to broad systemic modulation, given the distinct and sometimes opposing roles of receptor subtypes within the NVU (Cartier & Hla, 2019; Gaire, Song, et al., 2018; Hla & Brinkmann, 2011; Kim et al., 2015; Nitzsche et al., 2021). This is particularly evident when considering fingolimod, a non-selective S1P receptor modulator that has shown inconsistent results in preclinical and clinical studies of ischemic stroke (Brunkhorst et al., 2014; Ciubotaru et al., 2025; Czech et al., 2009; Kraft et al., 2013). These observations suggest that the therapeutic effects of fingolimod in ischemic stroke are highly context- and time-dependent. While fingolimod treatment during the early inflammatory phase has been associated with beneficial effects (Fu et al., 2014), delayed treatment or altered vascular conditions have been shown to reduce its therapeutic benefit and potentially compromise BBB integrity (Cantalupo et al., 2017; Li et al., 2020). This highlights the importance of temporal dynamics and vascular context in S1P targeted therapies. Our findings highlight a distinct spatiotemporal expression of S1PR3 within BBB cell populations during the acute phase of ischemic stroke. This suggests that timely and cell-specific targeting, rather than systemic modulation, might offer the potential to improve stroke outcome.

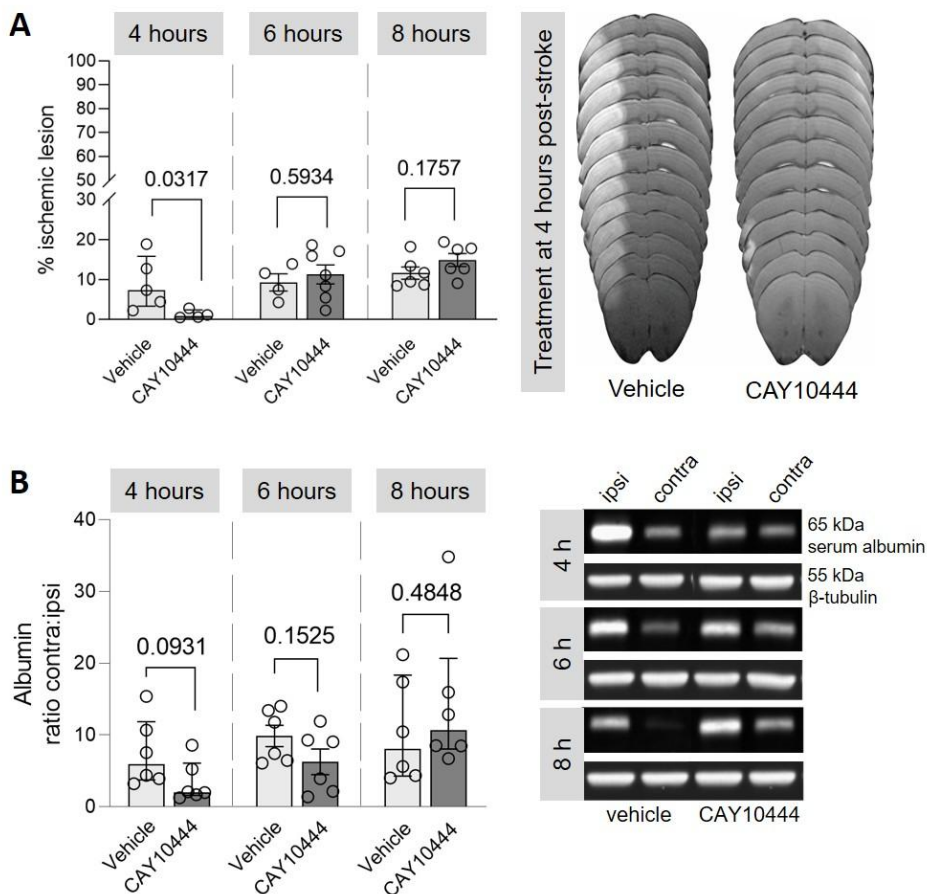


Figure 8| S1PR3 antagonism reduces infarct size and blood-brain barrier leakage in a time-dependent manner.

(A) Infarct lesion size 3-days after permanent middle cerebral artery occlusion (pMCAo) following sphingosine-1-phosphate receptor 3 (S1PR3) antagonist CAY10444 injections at 4, 6 or 8 h post-stroke. Quantification was performed using T2-weighted images (representative images for 4 h treatment and vehicle group are shown). (B) Serum albumin accumulation in parenchymal fraction 3-days post-stroke in vehicle and CAY10444-treated mice across different treatment timepoints (representative immunoblots are shown). Data are mean $\pm$ SEM (6 h) or median $\pm$ interquartile range (4 h, 8 h) and analyzed by Student's t-test (6 h) Mann-Whitney test (4 h, 8 h). Adapted from Matuskova et al., 2024.

Cortical S1P Signaling and Microglial Changes Under HFD Feeding (**Paper III**)

Given the increasing prevalence of obesity (World Health Organization, 2022) and its association with chronic low-grade inflammation, vascular dysfunction, and increased risk of neurological complications (Hotamisligil, 2006; Rohm et al., 2022), we investigated the role of S1P signaling in DIO. Previous studies have shown that genetic deletion of the S1P-generating enzyme *Sphk2* leads to protection against age-related obesity and insulin resistance (Ravichandran et al., 2019), and attenuates weight gain under HFD exposure (Zhao & Lee, 2023). Therefore, we investigated the effects of pharmacological SphK2 inhibition on S1P signaling and neuroinflammatory responses in the brain.

HFD exposure did not alter cortical *Sphk1* or *Sphk2* expression compared to CD-fed mice (**Fig. 9A**). However, consistent with earlier studies demonstrating that metabolic stress enhances sphingolipid signaling in the brain (Skoug et al., 2024), cortical S1P levels were increased following 9 weeks of HFD feeding (**Fig. 9B**). This elevation was accompanied by downregulation of *S1pr1* expression (**Fig. 9C**), and an increase in phosphorylated S1PR1 (pS1PR1; **Fig. 9D**), suggesting receptor desensitization and internalization in response to sustained ligand exposure (Chavez et al., 2015). Together, these findings indicate that HFD alters cortical S1P signaling dynamics, characterized by ligand accumulation and adaptive receptor regulation. Although *Sphk2* expression remained unchanged under HFD exposure, compensatory enzymatic activity changes cannot be excluded, as post-transcriptional regulation was not assessed and regional and cellular heterogeneity of lipid metabolism within the brain have been described (Bhaduri et al., 2021; Fitzner et al., 2020).

At the cellular level, HFD was associated with morphological changes in cortical microglia, including a reduction in endpoint number and total branch length per cell (**Fig. 9E-G**), indicating a less ramified phenotype that has previously been associated with reactive microglia and a shift away from homeostatic immune surveillance (Petry et al., 2023; Wang & Li, 2021). Notably, pharmacological inhibition of SphK2 restored microglial process complexity under HFD conditions (**Fig. 9F**), suggesting a link between S1P signaling and microglial morphology. Interestingly, this effect occurred without a detectable reduction in cortical S1P levels after SphK2 inhibition (**Fig. 9B**), pointing towards compensatory mechanisms or localized regulation of S1P signaling rather than global changes in lipid abundance. Overall, these findings describe how diet-induced metabolic stress modulates cortical S1P levels and microglial morphology, suggesting a link between sphingolipid metabolism and neuroinflammatory processes in obesity.

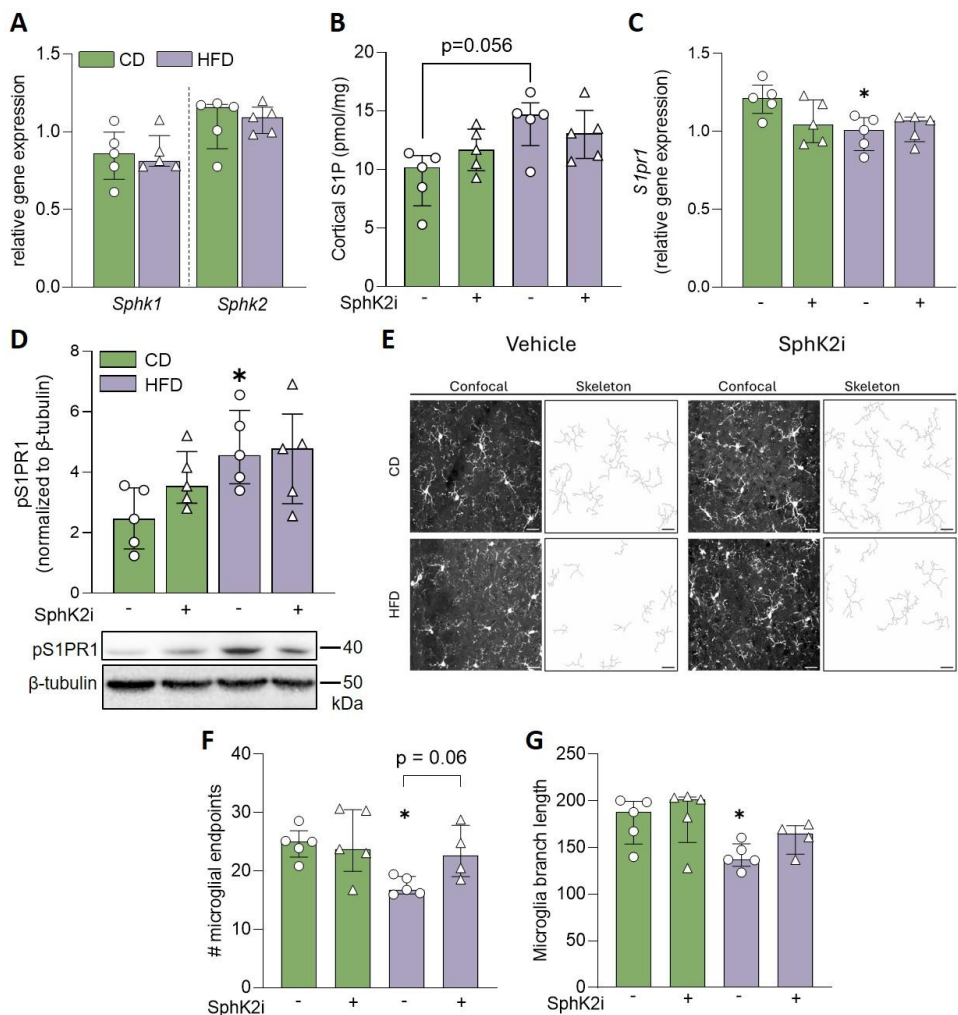


Figure 9| Diet-dependent regulation of cortical S1P signaling and microglial morphology.

(A) Cortical sphingosine kinase (*Sphk1* and *Sphk2*) expression in mice fed control diet (CD) or high-fat diet (HFD). (B) Cortical sphingosine-1-phosphate (S1P) levels in CD- and HFD-fed mice following treatment with SphK2 inhibitor (SphK2i) or vehicle. (C) Cortical S1P receptor (*S1pr1*) expression levels. (D) Cortical protein expression of phosphorylated S1PR1 (pS1PR1) levels (representative immuno blots are shown). (E) Representative confocal images of *Iba1*⁺ cortical microglia and corresponding skeletonization for analysis of number of endpoints and branch length (scale bar: 20 μ m). (F, G) Quantification of number of cortical microglial endpoints (F) and average branch length per cell (G) across diet and treatment groups. $n = 5$ for CD, CDi, HFD; $n = 4$ for HFDi. Data are median $\pm$ interquartile range (A - D, F, G) and analyzed by two-way ART-ANOVA followed by Mann-Whitney multiple comparisons or unpaired t-test (A). * $P < 0.05$ compared to corresponding CD group. Adapted from Vanherle et al., 2025.

HFD alters Post-stroke S1P Signaling and Attenuates S1PR3 Antagonism Treatment after Stroke (**Paper IV**)

Based on the beneficial effects of S1PR3 antagonism observed in healthy mice (Matuskova et al., 2024), we next wanted to extend these findings to a DIO model. Considering the role of metabolic status in stroke pathophysiology (Neeland et al., 2018), we therefore investigated how DIO affect systemic and cerebral S1P signaling. Under 8 weeks of HFD feeding, circulating S1P levels progressively increased over time (**Fig. 10A**), consistent with previous reports linking obesity to elevated systemic S1P (Kowalski et al., 2013). In parallel, baseline expression of S1P receptors was altered, specifically *S1pr2* and *S1pr5* were differentially expressed in both CD and HFD-fed mice compared to RCD controls (**Fig. 10B**), indicating that diet composition alone can shape cerebral S1P signaling. Purified diets differ from standard chow diet in composition and thereby their effects on the microbiota (González-Blázquez et al., 2020; Kang et al., 2013; von Süßkind-Schwendi et al., 2023; Warden & Fisler, 2008), influencing inflammation, membrane lipid composition, and sphingolipid metabolism (Maceyka & Spiegel, 2014; Norris & Blesso, 2017).

Following ischemic stroke, the previously observed reduction in plasma S1P in metabolically healthy mice was blunted in both CD- and HFD-fed mice (**Fig. 10C**). This suggests that prior diet exposure modifies systemic S1P responses to ischemic injury. Transcriptional analysis revealed dynamic, time-dependent changes in S1P receptor expression in HFD-fed mice after stroke (**Fig. 10D**), identifying *S1pr2* and *S1pr3* as the S1P receptors most strongly affected in response to ischemic injury.

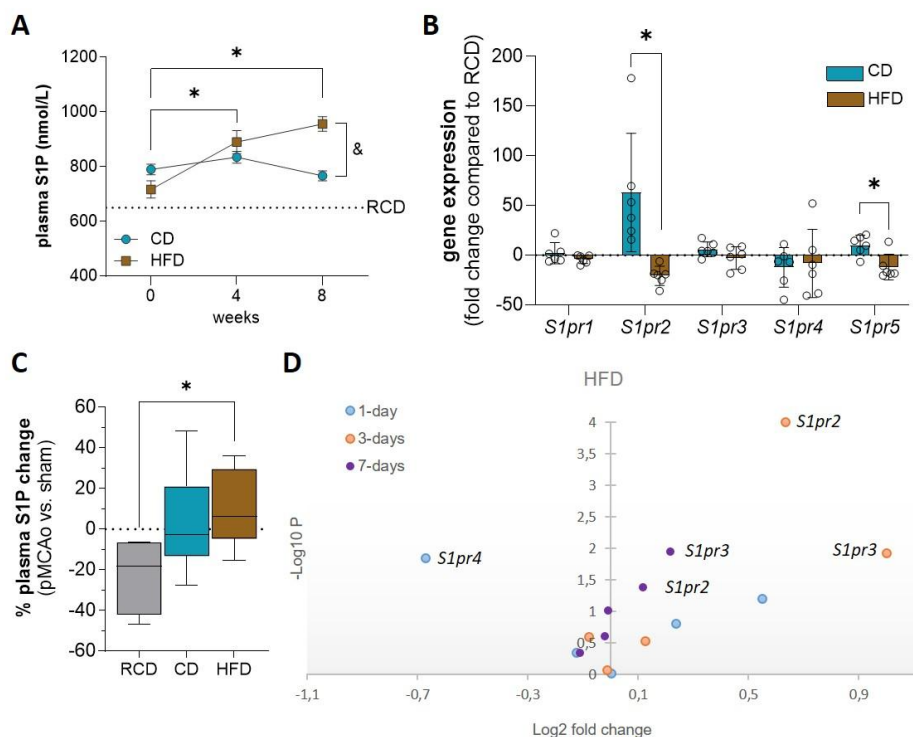


Figure 10| Diet-dependent modulation of baseline and post-stroke S1P signaling dynamics.

(A) Plasma sphingosine-1-phosphate (S1P) levels at baseline (0 weeks), 4 and 8 weeks of diet feeding. (B) S1P receptor (*S1pr*) gene expression in mice fed control diet (CD) and high-fat diet (HFD), shown as fold change compared to rodent chow diet (RCD). (C) Plasma S1P changes following permanent middle cerebral artery occlusion (pMCAo), normalized to respective sham-operated mice after diet feeding (RCD, CD or HFD). (D) Volcano plot of time-dependent (1-, 3- or 7-days post-pMCAo) gene expression changes in ipsilateral compared with contralateral hemisphere in HFD-fed mice after stroke. Data are mean $\pm$ SEM (A, B) and analyzed by 2-way ANOVA with Tukey's (A) or Sidak's (B) post-hoc or Kruskal-Wallis test with Dunn's multiple comparisons test (C) or paired t-test (D). *P < 0.05 for comparisons between timepoints, ^aP < 0.05 for comparisons between CD and HFD. For all others: *P < 0.05. Adapted from Paper IV.

Among all S1P receptors, *S1pr3* showed pronounced temporal upregulation, emerging as the most stroke-responsive receptor, with a particularly strong response at 3-days post-stroke (**Fig. 11A**). This pattern paralleled increased expression of *Gfap* (**Fig. 11B**), supporting a link between S1PR3 signaling and astrocyte activation, previously described in metabolically healthy mice after stroke (Matuskova et al., 2024). Consistent with earlier findings in metabolically healthy mice, S1PR3 antagonism reduced lesion expansion when administered early after stroke. However, its efficacy was attenuated in HFD-fed mice (**Fig. 11C**), despite comparable initial lesion sizes between RCD and HFD in vehicle-treated mice (**Fig. 11D**).

The reduced therapeutic responsiveness in DIO likely reflects broader alterations in S1P signaling, including increased ligand availability, altered receptor expression, and potential receptor desensitization (Gatfield et al., 2014; Mullershausen et al., 2009; Reeves et al., 2016; Skoug et al., 2024; Vanherle et al., 2025; Xu et al., 2022). Such context-dependent changes may shift the contribution of S1PR3 signaling to post-stroke injury and repair, thereby limiting the effectiveness of the used pharmacological treatment regimen. Together, these findings demonstrate that both diet composition and metabolic status reshape S1P signaling before and after ischemic stroke. Importantly, they highlight that metabolic context not only modifies neurovascular and inflammatory responses, but also influences responsiveness to S1P-targeted therapies, with implications for their clinical translation.

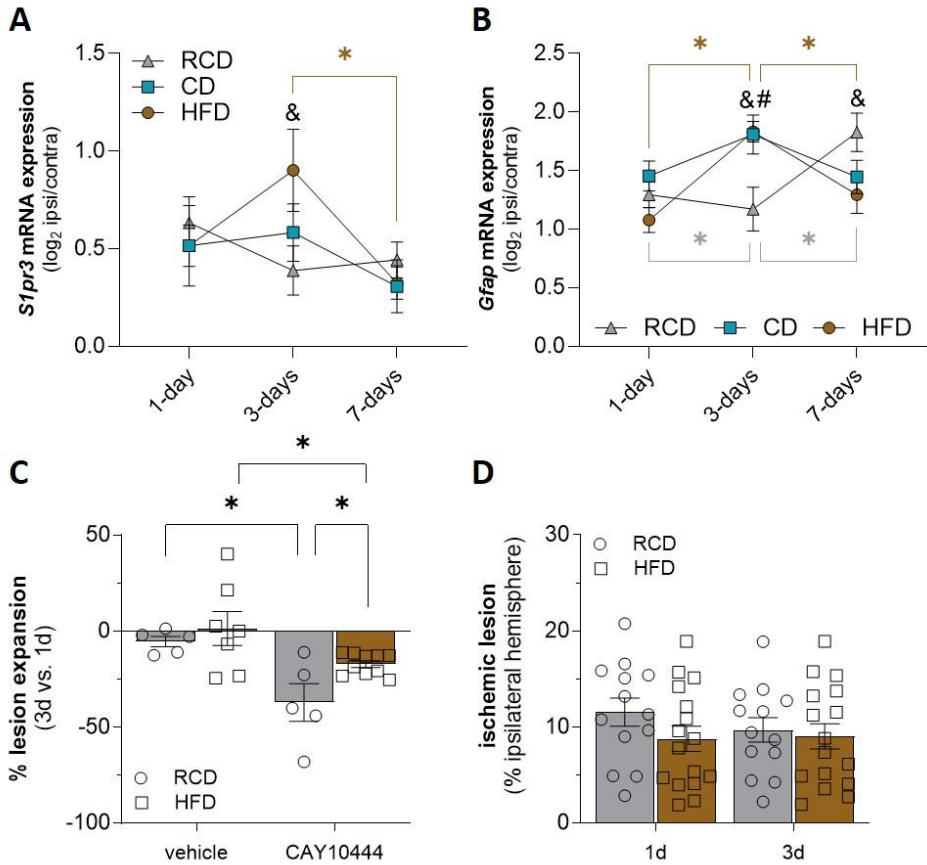


Figure 11| Diet-dependent temporal regulation of S1P signaling and neurovascular inflammation responses after stroke, with limited efficacy of S1PR3 antagonism in HFD-fed mice.

(A, B) Temporal expression of sphingosine-1-phosphate receptor 3 (*S1pr3*) (A) or glial fibrillary acidic protein (*Gfap*) (B) at 1-, 3- or 7-days after permanent middle cerebral artery occlusion (pMCAo) in mice fed rodent chow diet (RCD), control diet (CD) or high-fat diet (HFD)-fed. (C) Ischemic lesion expansion between 1-day (1d) and 3-days (3d) post-pMCAo following S1PR3 antagonist CAY10444 administered 4-hours post-pMCAo in RCD- or HFD-fed mice. (D) Infarct size at 1d and 3d post-pMCAo in vehicle-treated RCD- and HFD-fed mice. N = 7-11 per group (A, B); N = 5 (RCD) and N = 7-10 HFD (C); N = 13 RCD and N = 16 HFD (D). Data are mean $\pm$ SEM and analyzed by two-way ANOVA with Tukey's post-hoc testing (A, B) or Fisher's LSD post-hoc testing (C, D). *P < 0.05 for comparisons between days; &#P < 0.05 for comparisons between diet groups (RCD vs HFD). For all others: *P < 0.05. Adapted from Paper IV.

Conclusions and Future Perspectives

In this thesis, we investigated the role of S1P signaling in ischemic stroke and DIO, providing new insights into S1P pathway regulation and its implication in stroke pathology, obesity, neurovascular responses, and therapeutic targeting. Our findings identify S1P signaling as a central, context-dependent regulator of BBB function in ischemic stroke, influenced by both cellular compartmentalization and systemic metabolic status.

In **Paper I**, we demonstrated that plasma S1P levels are acutely reduced in both patients with ischemic stroke and murine stroke mouse models. This reduction was in parallel with altered endothelial S1P pathway expression and endothelial activation- and barrier-related markers. Notably, we identified an inverse correlation between plasma P-selectin and S1P levels in human ischemic stroke and model-dependent changes in plasma P-selectin in mice, suggesting a link between S1P depletion and expression of endothelial activation markers.

However, these findings rely largely on correlative and transcriptional readouts, and direct links between reduced S1P and endothelial dysfunction require further investigation. Future studies should focus on S1P restoration approaches, endothelial-specific SphK manipulation, and receptor-targeted rescue strategies (e.g., S1PR1 activation). Moreover, direct assessment of S1P metabolic flux and enzyme activity, as well as source-specific lipid analyses of circulating S1P pools (e.g., erythrocyte-, platelet-, or HDL-associated S1P), will be essential to better understand the physiological relevance of these changes.

In **Paper II**, we identified a critical role for S1PR3 signaling during the acute phase of ischemic stroke. Both genetic deletion and pharmacological antagonism of S1PR3 improved stroke outcome, as demonstrated by reduced infarct size, improved CBF, and attenuated BBB disruption when administered within an early therapeutic window. Importantly, elevated vessel-associated S1PR3 expression was observed following ischemia, while vessel-associated astrocytes were identified as the main S1PR3-expressing cell-population in the NVU. These findings suggest cell-specific targeting of S1P signaling as a promising therapeutic strategy for the treatment of acute ischemic stroke.

In **Paper I** and **II** the human stroke cohort used in the studies consisted mainly of patients with mild stroke phenotypes. Therefore, it might be of interest to explore and verify our findings in larger cohorts including patients with more severe stroke

cases and different stroke subtypes to strengthen the clinical translatability of our findings.

In **Paper III**, we investigated the contribution of S1P generation to DIO-associated neuroinflammation. DIO induced region-specific alterations in S1P signaling, with increased cortical S1P levels, reduced *S1pr1* expression, and enhanced receptor phosphorylation, while hippocampal S1P signaling remained largely unchanged. Furthermore, pharmacological inhibition of S1P generation via SphK2 attenuated microglial activation and partially restored microglial arborization and branch length in the cortex. These findings suggest that SphK2 plays a critical role in mediating DIO-associated neuroinflammation with more pronounced changes in the cortex.

Future studies should focus on the mechanistic interplay between S1P signaling, microglial activation, and cytokine pathways, ideally using more sensitive approaches to assess inflammatory responses beyond mRNA expression levels.

In **Paper IV**, we showed that diet and metabolic status influence both baseline and post-stroke S1P signaling dynamics. DIO progressively increased plasma S1P levels and altered cerebral S1P receptor expression, particularly *S1pr2* and *S1pr5*. Importantly, the observed reduction in brain and plasma S1P levels post-stroke in metabolically healthy mice was blunted with DIO. Furthermore, *S1pr3* expression showed a distinct temporal expression profile and altered *Gfap* expression. Functionally, pharmacological S1PR3 antagonism showed reduced responsiveness in DIO, indicating that metabolic context impacts therapeutic efficacy. This suggests that changes in ligand availability and receptor expression collectively contribute to treatment outcomes, emphasizing the importance of context-dependent therapeutic strategies.

Beyond the findings presented here, additional comorbidities such as hypertension may further influence S1P signaling in stroke and are therefore of interest for future investigations. Moreover, future studies should incorporate aged animals and both sexes, as sex-specific and age-dependent differences in stroke and metabolic disease are highly relevant for clinical translation.

Importantly, our work also highlights the often-overlooked importance of diet composition in experimental settings. Even moderate differences between standard chow and purified diets affected S1P signaling and stroke responses, underscoring the need for careful reporting and interpretation of dietary conditions in preclinical research.

In summary, this thesis demonstrates that: (1) S1P depletion is a hallmark of acute ischemic stroke and is paralleled by increased expression of endothelial activation markers and impaired BBB integrity, (2) S1PR3 represents a functionally relevant and targetable pathway in the acute phase of ischemic stroke, (3) S1P signaling is region-specific altered in the brain of DIO mice, with SphK2 playing a role in

neuroinflammatory responses under metabolic stress and (4) diet and metabolic status critically modifies S1P signaling and therapeutic responsiveness to S1PR3 antagonism post-stroke, limiting the efficacy of S1P-targeted interventions in obesity.

Together, these findings underscore the complexity of S1P signaling in stroke and obesity and highlight the importance of incorporating metabolic context, cellular and compartment specificity, and temporal dynamics in the development of future therapeutic strategies for ischemic stroke.

Artificial Intelligence (AI) Tools

During the writing process of this thesis, OpenAI's ChatGPT (GPT-5) and the AI tool Microsoft 365 Copilot (Version: 2.20260602.20.0) were utilized with the purpose of improving language grammar, overall clarity, and style of text as well as text readability and flow. All AI-generated suggestions were critically revised, edited and incorporated by this author, who takes full responsibility for the accuracy, integrity and originality of the written content of this thesis.

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