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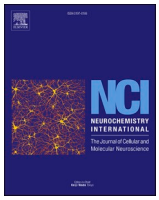

Unlocking the potential of low-molecular-weight (Poly)phenol metabolites: Protectors at the blood-brain barrier frontier

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ABSTRACT

Neurodegenerative diseases (NDDs) are an increasing group of chronic and progressive neurological disorders that ultimately lead to neuronal cell failure and death. Despite all efforts throughout decades, their burden on individuals and society still casts one of the most massive socioeconomic problems worldwide.

The neuronal failure observed in NDDs results from an intricacy of events, mirroring disease complexity, ranging from protein aggregation, oxidative stress, (neuro)inflammation, and even blood-brain barrier (BBB) dysfunction, ultimately leading to cognitive and motor symptoms in patients. As a result of such complex pathobiology, to date, there are still no effective treatments to treat/halt NDDs progression.

Fortunately, interest in the bioavailable low molecular weight (LMW) phenolic metabolites derived from the metabolism of dietary (poly)phenols has been rising due to their multitargeted potential in attenuating multiple NDDs hallmarks. Even if not highly BBB permeant, their relatively high concentrations in the bloodstream arising from the intake of (poly)phenol-rich diets make them ideal candidates to act within the vasculature and particularly at the level of BBB.

In this review, we highlight the most recent - though still scarce - studies demonstrating LMW phenolic metabolites' ability to modulate BBB homeostasis, including the improvement of tight and adherens junctional proteins, as well as their power to decrease pro-inflammatory cytokine secretion and oxidative stress levels *in vitro* and *in vivo*. Specific BBB-permeant LMW phenolic metabolites, such as simple phenolic sulfates, have been emerging as strong BBB properties boosters, pleiotropic compounds capable of improving cell fitness under oxidative and pro-inflammatory conditions. Nevertheless, further studies should be pursued to obtain a holistic overview of the promising role of LMW phenolic metabolites in NDDs prevention and management to fully harness their true therapeutic potential.

1. Introduction

In the corridors of modern medicine, a silent epidemic has been rising; one that imperceptibly steals away the essence of individuals, leaving behind a trail of cognitive decline and shattered lives – neurodegenerative diseases (NDDs). NDDs like Alzheimer's (AD) and Parkinson's disease (PD) are progressively debilitating conditions that lead to cognitive and motor dysfunction (Poewe et al., 2017; Ou et al., 2021; Muñoz-Delgado et al., 2023), posing a significant socioeconomic burden globally. These diseases share common pathological processes (Jain et al., 2023), including protein aggregation, oxidative stress, blood-brain barrier (BBB) dysfunction, and (neuro)inflammation (Poewe et al., 2017; Ou et al., 2021; Muñoz-Delgado et al., 2023). With effective treatments still elusive, research is increasingly focusing on the potential of diet, particularly plant-based foods, which are rich in (poly)phenols - bioactive **pleiotropic compounds** - to influence neurological health, mainly at prodromal stages, where significant inflammatory changes have already taken place (Terkelsen et al., 2022). Epidemiological studies suggest that long-term consumption of (poly)phenol-rich foods - such as fruits, vegetables, tea, and red wine - may reduce the risk of chronic diseases like cardiovascular issues and cancer (Medina-Remón et al., 2015; Carecho et al., 2023; Bianchi et al., 2023;

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Vitelli-Storelli et al., 2020), and may also slow cognitive decline, improve memory and learning, and reduce the risk of dementia and other NDDs (Devore et al., 2012; Lamport et al., 2016; Krikorian et al., 2010; Boespflug et al., 2018; Ng et al., 2008; Nurk et al., 2009). Higher intake of flavonoids, for instance, has been linked to lower odds of subjective cognitive decline and reduced mortality in PD patients (Zhang et al., 2022a, 2022b), underscoring the role of dietary (poly) phenols in mitigating the progression and impact of these devastating diseases.

Therefore, the prospect of modulating multiple hallmarks of NDDs through diet emerges as a promising non-invasive prevention strategy. And, indeed, both dietary (poly)phenols or their circulating metabolites will ultimately be differently bioavailable in the bloodstream. Indeed, individuals can exhibit variability in their ability to absorb dietary (poly)phenols, influenced by factors such as age, gender, gut health, and specific transporters and enzymes (Scalbert et al., 2005; Del Rio et al., 2012), as well as how (poly)phenols are consumed (raw vs. cooked) and overall diet composition, such as the presence of fat (Manach et al., 2004). The gut microbiota's role in metabolizing (poly)phenols into more bioactive metabolites also varies among individuals, affecting the compounds' biological activity (Williamson et al., 2010). These variations will certainly affect the neuroprotective effects and effectiveness in preventing NDDs (Rodriguez-Mateos et al., 2014). Nevertheless, even if (poly)phenol metabolites cannot cross the BBB and reach the brain in considerable concentrations, they will be bloodstream bioavailable and may still act effectively at the vascular and BBB endothelial levels.

In this review, we summarize currently known nutritionally relevant (poly)phenol metabolites' effects on BBB homeostasis and function, particularly against inflammatory conditions observed across NDDs like AD and PD.

2. Blood-brain barrier (BBB): key along NDDs progression

Before disclosing (poly)phenol metabolites' potential at BBB level, it is paramount to stress the importance of BBB for NDDs. The brain, a metabolically demanding organ, relies on a delicate nutrient balance to function optimally. The neurovascular unit (NVU), a dynamic structure in the microvasculature of the central nervous system (CNS), comprising the interplay between brain microvascular endothelial cells (BMEC, *i.e.*, the anatomic basis of the BBB), pericytes, astrocytes, neurons, and microglia, safeguards brain homeostasis, where BBB limiting most drugs' brain access, contributes to rendering NDDs untreated (Figueira et al., 2017a).

The BBB is a highly specialized capillary structure presented in all organisms with a well-developed CNS, one of the main evolutionary pressures that lead to the maintained homeostasis of the neuronal microenvironment. Classically, the BBB is mainly composed of BMEC, pericytes and astrocytes, characterized by sparse pinocytic vesicles, abundant mitochondria, and tight junctions (TJs) and adherens junctions (AJs) established between BMEC (Figueira et al., 2017a; Cardoso et al., 2010). While the BBB's low pinocytic activity limits most transcellular transport, the barrier permeability can be modulated through the regulation of TJ proteins, such as claudin and occludin, anchored to BMEC via scaffolding proteins from the zonula occludens (ZO) family, and of AJ proteins, like cadherins, cell adhesion molecules (CAMs) and junctional adhesion molecules (JAMs) (Abbott et al., 2010). Therefore, the expression and cytoskeleton organization of TJ and AJ proteins is closely linked to BBB permeability: increased occludin expression has been demonstrated to decrease BBB permeability by reducing paracellular transport (Balda et al., 2000), and elevated claudin-5 expression leads to decreased transit of large molecules (Nitta et al., 2003), while occludin deficiency has been shown to be directly related to BBB integrity impairment (Sugiyama et al., 2023).

More recently, a new terminology for BBB has been proposed, namely the concept of "blood-brain border", to reflect a more flexible and dynamic structure and function (Badaut et al., 2024). The term "blood-brain barrier" has been used through decades to emphasize restrictiveness, often reducing the notion of BBB properties to TJs and AJs molecules physically sealing BMEC, rather than emphasizing the complexity of this biological interface regarding its selectivity and multiplicity of exchanges between the blood circulation and the brain parenchyma. Such dynamic interface between blood and brain is capable of paramount adaptations and plasticity, and significant variations in BMEC properties occur at different sites along the brain's blood vessels. Importantly, functional TJs and AJs do not only secure proper sealing of the paracellular space between BMECs, but they are also key for the establishment of a polarized, transporting phenotype (*i.e*, apico-basal polarity via selective distribution of specific lipid- and protein complexes to distinct sites at the cell surface), where the cells are oriented with their apical site of the plasma membrane toward the lumen ("free surface"), and the lateral domains are in close contact with neighbouring cells and basally adhere to other solid tissues (or parenchyma), only separated by a basement membrane (Lee et al., 2014).

Such fine-tuned complex system, responsible for safeguarding one of the human body's sanctuaries – the brain – is one of the most important lines of defense against external insults. Among the intricate complexity of factors, BBB dysfunction is thought to be associated with pathology and development of multiple cerebrovascular diseases, including stroke, traumatic brain injury, and NDDs. In fact, several studies have provided a link between AD, PD and others and the dysfunction of the BBB, still arising doubts about whether the disruption of this barrier is a consequence and later event, or if it is a contributing factor for disease upsurge/progression (Weiss et al., 2009; Moon et al., 2022). What is known is that, despite its restrictiveness, BMECs are the first in line to sense and respond to external insult arriving through the bloodstream though they are still unable to thwart immune cell brain infiltration as occurs in chronic inflammatory diseases and NDDs (Yang et al., 2020).

2.1. BBB cytoskeleton rearrangement in NDDs

The maintenance of the BBB integrity heavily depends on its cytoskeletal organization. Under non-stressing conditions, TJs and AJs proteins are anchored to the actin cytoskeleton of the BMECs allowing a stable assembly and function of the barrier (Alkhalifa et al., 2023). On the other hand, progressive degeneration is known to negatively impact the cytoskeleton through multiple mechanisms. In AD, it has been shown that the inefficient clearance of $A\beta_{42,}$ results in the decreased expression of TJs proteins, via the receptor for advanced glycation end products (RAGE). The interaction of RAGE and Aβ42, through the Ras homolog gene family, member A/Rho-associated protein kinase (RhoA/ROCK) pathway, disrupts zonula occludens 1 (ZO-1) expression, increasing barrier permeability and protein deposition in the brain (Park et al., 2017). Moreover, it was also shown that RhoA-GTP levels were enhanced in the brain capillaries of *5XFAD* mice, supporting the hypothesis that Aβ42 induces the activation of the RhoA signalling, mediating cytoskeleton rearrangement, and evidencing a possible correlation between RhoA activation and BBB disruption in AD (Park et al., 2017).

Another factor contributing to the cytoskeletal alterations at BBB level along NDDs is the aggregation and hyperphosphorylation of proteins, such as tau protein: it has been proposed to be a bidirectional relationship, with BBB dysfunction contributing to tau hyperphosphorylation and aggregation and vice-versa (Michalicova et al., 2020). In line with this, an *in vivo* study with *rTg4510* mice found that solely tau accumulation is capable of inducing BBB damage, with increased permeability, though the exact mechanisms behind this phenomenon are still unknown (Blair et al., 2015).

In studies using lipopolysaccharide (LPS), a glycoprotein commonly used to induce neuroinflammation in rodents, it was demonstrated that LPS can directly damage the BMECs, altering their permeability (Salkeni et al., 2009). Evidence points to the activation of the RhoA/ROCK signaling pathway as one of the main effectors for the

inflammatory-dependent TJs disruption: when active, this pathway increases the phosphorylation of the myosin light chains (MLCs) (Feng et al., 2018), a class of Ca^{2+}-binding proteins involved in muscle function and kinetics. Their phosphorylation by myosin light chain kinase (MLCK) facilitates muscle movement by the contraction of the perijunctional apical actomyosin ring (Yang et al., 2021). Moreover, this phosphorylation also promotes increased permeability of BMECs by activating their contractile mechanism, thereby widening the spaces between them. Additionally, the TJs protein occludin is also phosphorylated, further impacting BBB cytoskeleton organization. In that sense, a previous study has shown that inhibiting the activity of MLCK could ameliorate the increase in BBB permeability induced by stroke by preventing the activity of the endothelial contractile machinery (Bartels et al., 2008). Whether this mechanism can be targeted by (poly)phenol metabolites is yet to be unveiled.

2.2. Junctional proteins' changes in NDDs

As the BBB is a selectively permeable barrier capable of blocking neurotoxicants from the peripheral circulation into the brain and, contrarily, enabling the entrance of nutrients, it is due to the existence of TJs that this property is mainly assured (Lochhead et al., 2020). Consequently, the expression and functionality of these TJ proteins are frequently and classically used as indicators of BBB integrity (Knox et al., 2022). In cerebral pathologies, the expression of TJs is altered, affecting BBB integrity. For instance, BBB disruption is observed in neurological disorders such as multiple sclerosis (MS), stroke, AD, PD, epilepsy, and traumatic brain injury (Berndt et al., 2019).

As previously mentioned, Kook et al., discovered that low concentrations of $A\beta_{1-42}$ induced structural BBB alterations via receptor for advanced glycation end products (RAGE) and reduced the protein levels of claudin-5 and occludin (Kook et al., 2012): by employing a neutralizing antibody against RAGE, it effectively blocked $A\beta_{1-42}$-induced disruptions in ZO-1, findings which were confirmed also *in vivo*, suggesting that Aβ-RAGE interactions could lead to TJs alterations in the BBB (Kook et al., 2012). Other studies in mice have shown that increasing claudin-5 expression reduced BBB paracellular permeability (Nitta et al., 2003), while in a murine AD model, breaking down of TJs allowed the paracellular clearance of neurotoxic Aβ peptides across the BBB (Ito et al., 2010). These findings represent a promising therapeutic target for the improvement of BBB integrity, although, a more controlled and targeted modulation of TJs needs to be considered (Alkhalifa et al., 2023).

Concerning PD animal studies, a dysfunctional and compromised BBB has been shown upon treatment, using different methods, observed by the leakage of albumin and other tracers into the brain parenchyma (Carvey et al., 2005; Zhao et al., 2007; Chen et al., 2008; Carta et al., 2006), enhanced entry of drugs, and infiltration of peripheral immune cells (Carta et al., 2006). Moreover, such BBB impairment in PD animal models has also been observed by higher IgG leakage to the brain and loss of TJ proteins at BBB level: in a study using A53T PD mouse model, it was shown a decrease of ZO-1, claudin-5, and occludin proteins led to increased vascular permeability (Lan et al., 2022). Likewise, in PD patients, it was also reported the occurrence of BBB leakage by increased levels of CSF albumin, which correlated with disease severity (Pisani et al., 2012). Moreover, in living PD patients it was possible to identify increased uptake of ^{11}C-verapamil tracer in the midbrain using positron-emission tomography (PET), indicating BBB efflux pump P-glycoprotein (P-gp) impairment (Kortekaas et al., 2005; Bartels et al., 2008), while through imaging analysis of dynamic contrast-enhanced magnetic resonance, higher BBB leakage was observed as well in PD patients (Al-Bachari et al., 2020).

Beyond TJs, AJs are also implied in the development, stabilization, and organization of the intercellular junctions at the BBB endothelium (Knox et al., 2022). The vascular integrity maintenance of AJs is mainly assured by VE-cadherin, and its loss has been described to contribute to dysfunctional vascular integrity and the development of AD (Dejana et al., 2008; Li et al., 2020). On the other hand, restoring VE-cadherin expression levels can lead to disease regression (Qin et al., 2019). Also IL-1β-induced pericytes were shown to increase BBB permeability to sodium fluorescein (Na–F) leading to enlarged intercellular gap in human BMEC (Qin et al., 2019), mainly via damaging of TJs and AJs like VE-cadherin, which interacts with β-catenin to regulate endothelial cell-cell adhesions, and so the two are needed to maintain AJs homeostasis (Qin et al., 2019; Guo et al., 2008). In contrast, melatonin was able to reduce BBB permeability to Na–F by inhibiting junctional protein disruption (Qin et al., 2019).

While under normal conditions Wnt/β-catenin signalling is crucial for CNS angiogenesis (Liu et al., 2014a), in AD, a dysfunction of the Wnt/β-catenin signalling pathway occurs, leading to a decreased expression of TJs and a modulation of transporters expression, like P-gp and glucose transporter 1 (GLUT1), ultimately increasing BBB permeability (Liu et al., 2014a). It is acknowledged that suppressing Wnt/β-catenin signalling in the AD transgenic $APP_{swe}/PS1_{dE9}$ (APP/PS1) microvessels increased glycogen synthase kinase-3 beta (GSK-3β), whereas its activation restored the BBB TJs, and prevented Aβ-induced endothelial cells' dysfunction (Wang et al., 2022). In human iPSCs, it was also described that the activation of the Wnt/β-catenin pathway induced GLUT1 and claudin-5 expression (Gastfriend et al., 2021), and in adult mice with β-catenin endothelial-conditional knockout, a reduced TJs protein expression and increased caveolae-mediated transcytosis was observed (Alkhalifa et al., 2023; Hussain et al., 2022), a possible window of action where bioavailable phenolic metabolites can have a decisive role.

2.3. Adhesion molecules in NDDs

Adhesion also plays a crucial role in maintaining the integrity of the BBB and regulating leukocyte recruitment (Ma et al., 2013). In NDDs like PD, AD, and MS, adhesion molecules are altered at the level of the BBB contributing to its dysfunction and to the exacerbation of disease pathology (Yuan et al., 2023).

Adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) are overexpressed on endothelial cells in inflammatory conditions, central in NDDs (Alkhalifa et al., 2023; Zhang et al., 2023), and VCAM-1, much like ICAM-1, is a key mediator in the recruitment and transmigration of leukocytes across the BBB. It is implicated in MS, where its upregulation promotes the adhesion of lymphocytes to the endothelial cells of the BBB, enabling intravasation into the CNS (Boyce et al., 2002).

In AD patients, higher levels of VCAM-1 and endothelial (E)-selectin have been observed (Drake et al., 2021). Such observations have been shown to be correlated with dementia and cognitive decline, and tau/$A\beta_{1-42}$ ratio in CSF, respectively, indicative of vascular dysfunction. Soluble ICAM-1 and P-selectin – which is expressed on stimulated endothelial cells and activated platelets - levels are also increased in AD which, all together, are indicative of vascular inflammation and alteration in cell adhesion processes. Soluble platelet endothelial cell adhesion molecule 1 (PECAM-1) levels have also been reported to be higher in AD, mediating leukocyte migration during inflammation (Zenaro et al., 2017). Miklossy et al. in a human intervention study, found elevated levels of ICAM-1-positive glial cells in patients with PD (Miklossy et al., 2006), while Sharief et al. directly correlated BBB damage with increasing levels of ICAM-1 in serum and CSF in cases of MS (Sharief et al., 1993). As strong anti-inflammatory compounds in physiologically relevant concentrations (Carregosa et al., 2020), it is reasonable to foresee that both bioavailable dietary (poly)phenols and/or circulating (poly)phenol metabolites resulting from (poly)phenol-rich diets can have the potential to modulate these or other adhesion molecules at BMEC level and, consequently, modulating cell adhesion mechanisms, reducing brain cell infiltration and boosting barrier properties, altogether attenuating both systemic and neuroinflammation at NVU level.

2.4. Matrix metalloproteinases at BBB level in NDDs

Matrix metalloproteinases (MMPs) are zinc-dependent enzymes and the primary agents of extracellular matrix degradation and remodeling (Archie et al., 2021). Under pathological conditions, those molecules directly impact BBB integrity and dysfunction at the onset of different neurological disorders, mainly by degrading TJ proteins (Nichols et al., 2021; Zhang et al., 2021). For instance, MMP-9 production in the brain has been shown to lead to BBB impairment through rearrangement and degradation of TJ-associated proteins (Zhang et al., 2012; Bauer et al., 2010). In contrast, the inhibition of MMPs has been associated with ICAM-1 and VCAM-1 downregulation, which negatively impact inflammatory cell migration across the BBB (Archie et al., 2021; Lee et al., 2003).

In pericytes, the activation of the proinflammatory CypA-metallopeptidase 9 (MMP9) pathway has been shown to contribute to the breakdown of the BBB. NF-κB transcriptionally activates MMP9 in cerebral vessels, causing BBB dysfunction, via decreasing levels of claudin-5 (Holland et al., 2015). Previously, it has been shown that ApoEε4 stimulates the CypA-MMP9 pathway, which could result in the BBB breakdown that ultimately leads to neuronal and synaptic dysfunction; subsequently, it was reported that the blockade of the CypA-MMP9 pathway in ApoEε4 knock-in mice rectified BBB's integrity, by the re-expression of several TJs and AJs proteins, allowing for the restoration of neuronal and synaptic functions (Bell et al., 2012). Accordingly, targeting the CypA-MMP9 cascade could also represent another promising strategy for BBB maintenance (Kirchner et al., 2023): using SB-3CT, an MMP9 inhibitor, it was shown to eliminate the MMP9 gelatinase activity, reversing the BBB's leakiness. In addition, the genetic inhibition of the CypA-MMP-9 pathway at the BBB reversed neurodegenerative changes in Apoe$^{-/-}$ mice (Bell et al., 2012). Indeed, olive oil and red wine dietary (poly)phenols, oleuropein and hydroxytyrosol as well as resveratrol and quercetin, have been demonstrated to reduce inflammatory angiogenesis in cultured endothelial cells, through MMP-9 and cyclooxygenase (COX)-2 inhibition (Scoditti et al., 2012), paving the way for further investigation of bioavailable (poly)phenol metabolites towards these mechanisms at BBB level.

2.5. Oxidative stress at BBB level in NDDs

The term oxidative stress is a condition produced by the imbalance between oxidants and antioxidants in biological systems, a result of elevated levels of reactive oxygen species (ROS) or due to improper functioning of the antioxidant system (Scarian et al., 2024). In fact, inflammatory processes are closely linked to oxidative stress, with ROS promoting pro-inflammatory gene expression and, simultaneously, neuroinflammation enhancing ROS production. Under normal conditions, the inflammatory response serves as a defense mechanism, but in pathological states, redox imbalance triggers inflammatory mechanisms leading to pro-inflammatory molecules secretion, that further induce ROS generation in non-phagocytic cells, mainly via NADPH oxidase activation (NOX) (Scarian et al., 2024).

In NDDs, oxidative stress is a well-known factor involved in various neurodegenerative mechanisms, including oligomerization of proteins like alpha-synuclein (αSyn) and Aβ; cytokine production and inflammatory responses; and BBB disruption (Yaribeygi et al., 2018; Sienes Bailo et al., 2022; Tarozzi, 2020). ROS can modulate the expression of ZO-1, leading to compromised BBB integrity. Similarly, AMP-activated protein reduces occluding expression by suppressing NADPH oxidase activation (Song et al., 2020).

In AD, it is known that Aβ can induce oxidative stress and inflammation along with pericyte degeneration and BBB breakdown (Zenaro et al., 2017). Moreover, previous studies have shown that tau pathology disrupts BBB through mechanisms such as mitochondrial dysregulation, oxidative stress, and chronic neuroinflammation. Tau impacts mitochondrial localization, distribution, and dynamics, leading to changes in ATP levels and reactive oxygen species production (Tao, 2022). In PD, oxidative stress has also been reported to mediate BBB disruption; for instance, MMP-3 active form is increased in response to oxidative stress in dopaminergic cells, leading to microglia activation, reactive nitrogen species (RNS) and ROS production (Choi et al., 2014), BBB degradation, and ultimately neuroinflammation (Choi et al., 2011). In acute ischemic stroke, BBB breakdown occurs within hours of onset mainly due to oxidative stress and ROS, which affect TJ proteins and the cytoskeleton of BMEC and at later stages, neuroinflammation processes further exacerbate BBB disruption (da Fonseca et al., 2014).

In addition to the mechanism highlighted before underlying ROS/RNS-induced BBB disruption, also MMP appears to be a key mediator in the initiation and progression of BBB damage elicited by oxidative stress (Lehner et al., 2011). Several neuroprotective therapies have been developed to counteract ROS and protect neurons along NDDs. Identifying the precise mechanisms of ROS-induced neurodegeneration at the BBB level and cell death will be crucial for targeting effective treatments.

2.6. Pro-inflammatory mediators in NDDs

Besides oxidative stress, protein aggregation, or even cell death, (neuro)inflammation, a key hallmark of NDDs, greatly impacting BBB homeostasis (Takata et al., 2021). Neuroinflammation is often associated with BBB leakage, either from within the NVU or from peripheral sources (Huang et al., 2021).

BBB dysfunction is intimately related to microglia activation given that the released pro-inflammatory mediators by the glia culminate in endothelial alterations. The pivotal mediator of most inflammatory responses by the glia is the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which induces the expression of a variety of pro-inflammatory genes, including cytokines and chemokines-related genes. It has been shown that the overexpression of interleukins (IL-), such as IL-6 and tumor necrosis factor alpha (TNF- α) is a driving factor in the development of AD (Wu et al., 2015). Additionally, increased levels of IL-6 have also been reported to be accompanied by decreased levels of TJ and AJ (occludin, claudin-5 and VE-cadherin) (Argaw et al., 2012). In a BBB *in vitro* model using BMECs, both TNF-α and IL-1β impacted BBB permeability with increased expression of VCAM-1, ICAM-1 and NLRP3, and concomitant decrease of several TJ proteins (as ZO-1, claudin-3 and -5) (Versele et al., 2022). This finding is significant as it highlights the prominent role of inflammasome NLRP3 pathway for BBB homeostasis. In fact, NLRP3 pathway is intimately connected with the inflammatory response triggered by IL-1β, working in tandem with NF-κB, producing the mature form of IL-1β and IL-18. It has been shown that the activation of the NLRP3 inflammasome and the subsequent release of IL-1β are linked to the BBB damage and its increased permeability (Argaw et al., 2012).

Similarly, TGF-β also plays an important role in neuroinflammation: while it increases the paracellular permeability of vascular endothelial layers by influencing the tyrosine phosphorylation of VE-cadherin and claudin-5, it is also implicated in the deleterious cycle where a compromised BBB amplifies TGF-β signaling in astrocytes, which in turn exacerbates cognitive deficits in aging rodents (Shen et al., 2011). By counteracting IL-1β's effects, either by using IL-1β receptor antagonists or through the genetic removal of the IL-1 receptor, it has been shown that BBB's hyperpermeability induced by neuroinflammation can be mitigated (Zhang et al., 2018a).

Another prominent player in the inflammatory response at BBB level and beyond is the mitogen-activated protein kinase (MAPK) family of protein kinases. Comprised of the extracellular signal-regulated kinase (ERK), p38 and c-Jun N-terminal kinase (JNK), this pathway is triggered by pro-inflammatory cytokines, being a primary mediator for neuronal apoptosis (Lahiani et al., 2018). A study found that dexmedetomidine inhibits the activation of MAPK pathways in an induced-stroke rat model via the decreased phosphorylation of its main players, while the p38

MAPK inhibitor (SB203580) maintained the BBB integrity and attenuated the inflammatory response (Zhu et al., 2024).

Given the multifaceted nature of (neuro)inflammation and its deleterious impact on the BBB, either as a cause or a consequence of other NDDs hallmarks that range from protein aggregation to oxidative stress, lipid peroxidation, mitochondrial dysfunction, deficient clearance mechanisms, etc., it is expected that various inflammatory-dependent and independent mechanisms converge to alter its permeability and integrity. These mechanisms involve the interplay of pro-inflammatory cytokines and signaling pathways, which collectively disrupt TJs, AJs, the endothelial cytoskeleton, and adhesion but also produce more inflammatory molecules that further damage the BBB (Fig. 1). Nevertheless, the precise timeline and cascade of events that culminates with NDDs pathological manifestations are still not fully understood, seriously hampering the efficacy of novel therapeutics emerging every day. On the other hand, the (poly)phenol-rich diets and the modulation of the levels of the different strong anti-inflammatory pleiotropic (poly)phenol metabolites in circulation before NDDs development can represent a very promising roadmap and a shift in mentality in the way we tackle these uncurable disorders. But, to do so, we need to better understand which (poly)phenols arise from diet and metabolism, as well as their bioavailability in the bloodstream.

3. (Poly)phenols metabolism and bioavailability

For a full understanding of dietary (poly)phenols neuroprotective action at BBB level or even inside the brain in the scope of NDDs, it is imperative to consider their absorption, distribution, metabolism, and excretion (ADME) profile. In this regard, not only should they be considered but, perhaps more importantly, the metabolites that result from this ADME should be explored for their health potential (Carregosa et al., 2022).

The term "(poly)phenol" denominates a family of organic compounds characterized by the presence of aromatic rings and at least one hydroxyl group on said rings. Various plant-based dietary products, like fruits, vegetables, tea, and wine, are rich sources of these compounds. (Poly)phenols can be divided into several families, based on their chemical structure, including flavonoids, stilbenes, phenolic acids, and

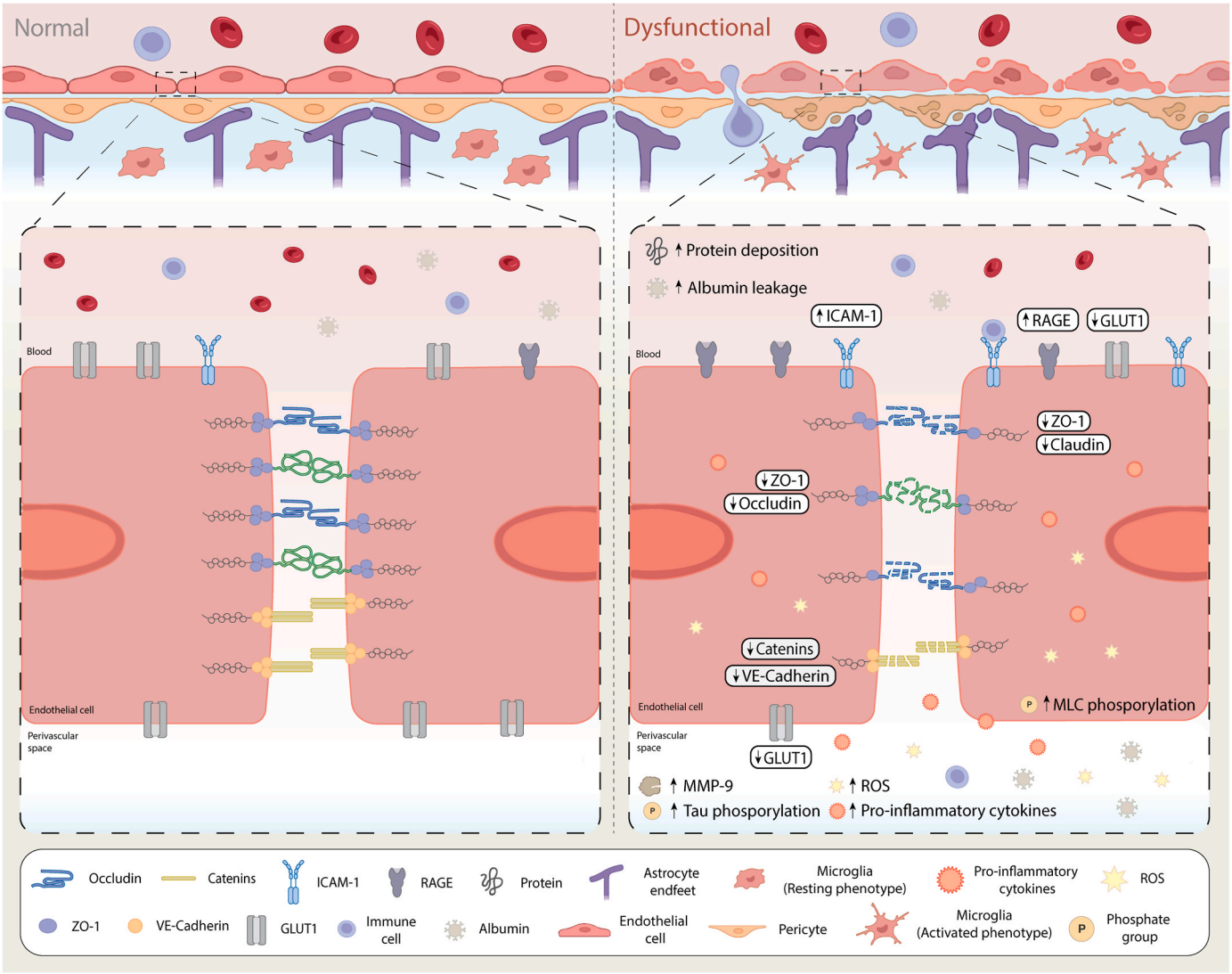

Fig. 1. Mechanisms of dysfunctional blood-brain barrier (BBB) induced by an inflammatory environment as it is observed in NDDs. In non-inflamed basal conditions (left side), there is a normal expression of membrane receptors and junctional proteins that safeguard the brain homeostasis. When an inflammatory process occurs (right side), the BBB integrity becomes compromised, leading to junctional proteins' disruption, cytoskeleton alteration, adhesion molecules modulation, cell infiltration, pro-inflammatory cytokines release, and misfolded proteins brain leakage. Abbreviations: zonula occludens 1 (ZO-1), receptor of advanced glycation end products (RAGE), glucose transporter 1 (GLUT1), intercellular adhesion molecule 1 (ICAM-1), metallopeptidase 9 (MMP9), myosin light chains (MLCs), reactive oxygen species (ROS).

lignans, among others (Figueira et al., 2017a; Carregosa et al., 2020). Among those, flavonoids and phenolic acids appear as the ones that seem to present major biological relevance, possibly due to their elevated concentration on dietary products and their higher bioavailability (Carregosa et al., 2020). Unfortunately, only a minor fraction of dietary (poly)phenols are absorbed, being extensively metabolized along the digestive tract, limiting their bioavailability and biodistribution (Carregosa et al., 2022).

Research suggests that some flavonoids and phenolic acids, like anthocyanins and gallic acid, may be absorbed directly in the stomach (Lewandowska et al., 2013; Pimpão et al., 2015; Passamonti et al., 2002); nevertheless, flavonoids exist mostly in a glycosylated form, reaching the small intestine, where the sugar attached is released by endogenic β-glucosidases (Lewandowska et al., 2013). What is important to stress is that the lipophilicity of the released aglycones is usually higher than the primary glycosides, enabling them to enter the epithelial cells via passive diffusion. In the enterocytes, (poly)phenols can undergo phase I biotransformation (oxidation, reduction, or hydrolysis) (Pimpão et al., 2015) and are then transported, mainly bound to proteins like albumin, via the portal vein to the liver where most of phase II metabolism takes place. Similar to phase I and other xenobiotics metabolism, phase II reactions will increment the solubility of the compounds and facilitate their elimination in bile and urine, an event comprising their conjugation with sulfate, methyl, and glucuronic acid (Lewandowska et al., 2013). (Poly)phenols not absorbed in the small intestine (90–95% of the initial intake) reach the large intestine in aglycone form or simply untouched. It's here that gut microbiota catabolizes them through ring fission, generating what is called the **low-molecular-weight (LMW) phenolic metabolites** (Carregosa et al., 2022). In the kidney and, parallelly, inside the entero-hepatic circulation, these LMW phenolic metabolites will be subjected to phase II metabolism, and be eliminated through urine or enter systemic circulation, respectively.

3.1. Low-molecular-weight (LMW) phenolic metabolites

Unfortunately, only 5–10% of dietary (poly)phenols are absorbed in the small intestine, whereas the great majority reaches the colon, being extensively metabolized by the gut microbiota into LMW phenolic metabolites (Carregosa et al., 2022; Kay et al., 2020). Fortunately, those compounds have been identified as the molecules responsible for health-beneficial effects of (poly)phenols consumption, some of them even related to brain health improvements (Carecho et al., 2022; Cortés-Martín et al., 2020; de Ferrars et al., 2014).

As previously mentioned, LMW phenolic metabolites appearance can be devoted to different pathways, either derived directly from the diet, catabolism by microbiota (*i.e.*, flavonols, flavones, anthocyanins, chalcones, chlorogenic acids, and others), from endogenous pathways, or even from the metabolism of industrial chemicals and pharmaceuticals (Carregosa et al., 2022). Apart from the gut catabolism origin of LMW phenolic metabolites, some of them may appear in circulation due to their presence already in the food matrix, as, for instance, 4′-hydroxy-3′-methoxycinnamic acid (ferulic acid) and 3′,4′-dihydroxycinnamic acid (caffeic acid) (Carregosa et al., 2022). In contrast, 3′,4′-dihydroxyphenylacetic acid (DOPAC) and 4′-hydroxy-3′-methoxyphenylacetic acid (homovanillic acid) are examples of LMW phenolic compounds that could be both endogenously produced and may result from microbiota catabolism (Carregosa et al., 2022).

Studies have shown that due to digestive, colonic, and hepatic processing, (poly)phenols are only detected at nanomolar concentration or even undetectable in the tissues and bloodstream (Lewandowska et al., 2013). On the contrary, it has been shown that after the consumption of (poly)phenol-rich foods or beverages, LMW phenolic metabolites, like simple phenolic sulfates, are found in circulation in concentrations several times higher than their parent counterparts, which can be explained, at least partially, by the fact that these metabolites can have their origin in different parent compounds (Carregosa et al., 2022; Pimpão et al., 2015). Importantly to stress, the composition of the gut microbiota varies on an inter-and intra-population basis, which also explains the individual variation in the bioavailability of (poly)phenols and originating LMW phenolic metabolites (Lavefve et al., 2020; Liu et al., 2010; Bolca et al., 2013). This way, when considering an exclusively nutritional approach, we can anticipate that the metabolites in circulation (alone or in combination with dietary (poly)phenols) can constitute the most promising leads to study as potential health-effector compounds.

Luckily, in recent years, a considerable amount of new data linking particular dietary patterns and beneficial effects in the brain preventing NDDs and cognitive decline has been emerging (Nooyens et al., 2011; Psaltopoulou et al., 2013). Greater intake of (poly)phenol-rich foods, such as fruits, coffee, olives and olive oil, red wine, tea, dark chocolate, nuts, vegetables, and spices, as is observed in Mediterranean Diet (MeDi) patterns have been associated with slower rates of cognitive decline, improved memory and learning performance, and even improved neuronal responses in humans (Devore et al., 2012; Lamport et al., 2016; Krikorian et al., 2010; Boespflug et al., 2018; Ng et al., 2008; Nurk et al., 2009). Indeed, MeDi is recognized as one of the healthiest dietary patterns, characterized among others by a higher (poly)phenol intake, consistently associated with several beneficial health outcomes (Mendonça et al., 2022). Compiling evidence stresses the impact that adherence to MeDi can hold in the prevention of NDDS, like PD (as reviewed in (Bisaglia, 2022)). Unfortunately, adherence to the MeDi pattern has been decreasing in southern European countries for the last decades, especially among low socioeconomic groups (Mendonça et al., 2022). Researchers have also established associations between higher intakes of flavonoid-rich diets with lower levels of inflammatory biomarkers (INVALID CITATION). How these differences in adherence to particular dietary patterns relate to particular LMW phenolic metabolites signatures and ultimate NDDs severity, is yet to be defined: we still lack a deep understanding if and how (poly)phenols consumption, LMW phenolic metabolites circulating levels, and NDDs severity/progression correlate. Indeed, most mechanistic studies conducted so far have been centred either on plant-extracts, without considering human metabolism, or have been focused on parent dietary (poly)phenols (*i.e.*, the ones found in food) that, unfortunately, reach very low concentrations in circulation (Carregosa et al., 2022). Moreover, studies that lean towards a more nutritional perspective prioritize the effect observed against neuroinflammation, oxidative stress, or even BBB improvement rather than identifying the LMW phenolic metabolite responsible for it. Nonetheless, the evidence presented by the parent (poly)phenol compounds, both in *in vitro* and *in vivo*, is extremely valuable for the general understanding of the involved mechanisms and it can also be used as a foundation for future case studies that focus primarily on the nutritional approach and LMW phenolic metabolites (Carregosa et al., 2020). Whilst, by opposition, despite more bioavailable, LMW phenolic metabolites are still marginally explored as the tangible link between nutrition and brain health across multiple model systems (Carregosa et al., 2021), particularly what is their role in halting the development of NDDs at BBB level.

3.2. LMW phenolic metabolites: BBB permeant pleiotropic leads

To correctly evaluate the neuroprotective role of (poly)phenols and their metabolites, is essential to understand and consider the capacity that LMW phenolic metabolites have at least to approach, or ideally permeate the BBB and ultimately reach the brain. As previously stated, the functionality of the BBB is severely affected in cases of chronic inflammation, like NDDs, in a way that its permeability rises, allowing a freer solute/macromolecule diffusion and the infiltration of pro-inflammatory cells and/or pathogenic microorganisms. Having this in mind, it is easy to comprehend the potential protective role of highly bioavailable LMW phenolic metabolites at vascular and BBB levels, whether they can be transported across the BBB endothelium or not

(Carecho et al., 2021).

Previous studies presented the notion that the BBB permeability of a compound depends on its lipophilicity, where polar metabolites, like sulfate and glucuronide conjugates, may have reduced permeability when compared to less polar, methylated conjugates (Youdim et al., 2003, 2004; Shimazu et al., 2021; Gasperotti et al., 2015). Simple phenolic sulfates, when tested in circulating concentrations, have been shown to be transported across the BBB endothelium, presenting strong anti-inflammatory and neuroprotective potential, both at neuronal and at BBB level (Figueira et al., 2017b), reinforcing the hypothesis that bioavailable LMW phenolic metabolites can indeed comprise strong candidates in preventing NDDs. More recently, such observations were validated *in vitro* and *in vivo,* and the BBB transport and brain uptake of these LMW phenolic metabolites appeared to be true for an even shorter time window (Carecho et al., 2024). Remarkably, these LMW phenolic metabolites also boosted BBB properties, modulating junctional proteins' expression and subcellular localization (Carecho et al., 2024) (Fig. 2). Additionally, some of these BBB-permeant LMW phenolic metabolites' appearance and quantification in the human CSF of healthy subjects have been reported as well (Le Sayec et al., 2023), compiling evidence that supports our hypothesis of these metabolites as brain-accessible compounds and potential key players in NDDs prevention. Either way, the main molecular events associated with the true BBB uptake of LMW phenolic metabolites, being passive diffusion or carrier-mediated transport, remain unclear (Carecho et al., 2021), and further studies are necessary to clearly elucidate it, hoping to fill the knowledge gap between (poly)phenol intake, brain bioavailability and neuroprotective potential.

Besides their BBB uptake, also some data has been emerging regarding the effects of LMW phenolic metabolites towards diverse NDDs hallmarks, particularly against (neuro)inflammation. Several *in vitro* studies have begun to elucidate the mechanisms behind their anti-inflammatory properties, showing an association between dietary (poly) phenols consumption and a reduction in inflammatory biomarkers (as previously reviewed (Carregosa et al., 2020)). Current data on LMW phenolic compounds mechanisms of action indicate several main targets in microglia cells, ranging from the inhibition of the activation of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), iNOS induction and NO production, inhibiting COX-2 expression, and downregulating pro-inflammatory transcription factors such as NF-κB through modulation of glial and neuronal signaling pathways (Carregosa et al., 2020). As for neurodegeneration, some more advanced 3D cellular systems have already been employed to disclose some phenolic sulfates' potential against oxidate stress and dopaminergic cell death *in vitro*, pointing to the modulation of anti-apoptotic cell machinery and glutathione metabolism (Carecho et al., 2022; Figueira et al., 2017b). In rodent models of NDDs, beneficial effects against pro-inflammatory markers, oxidative stress, lipid peroxidation, and even Aβ deposition have been reported

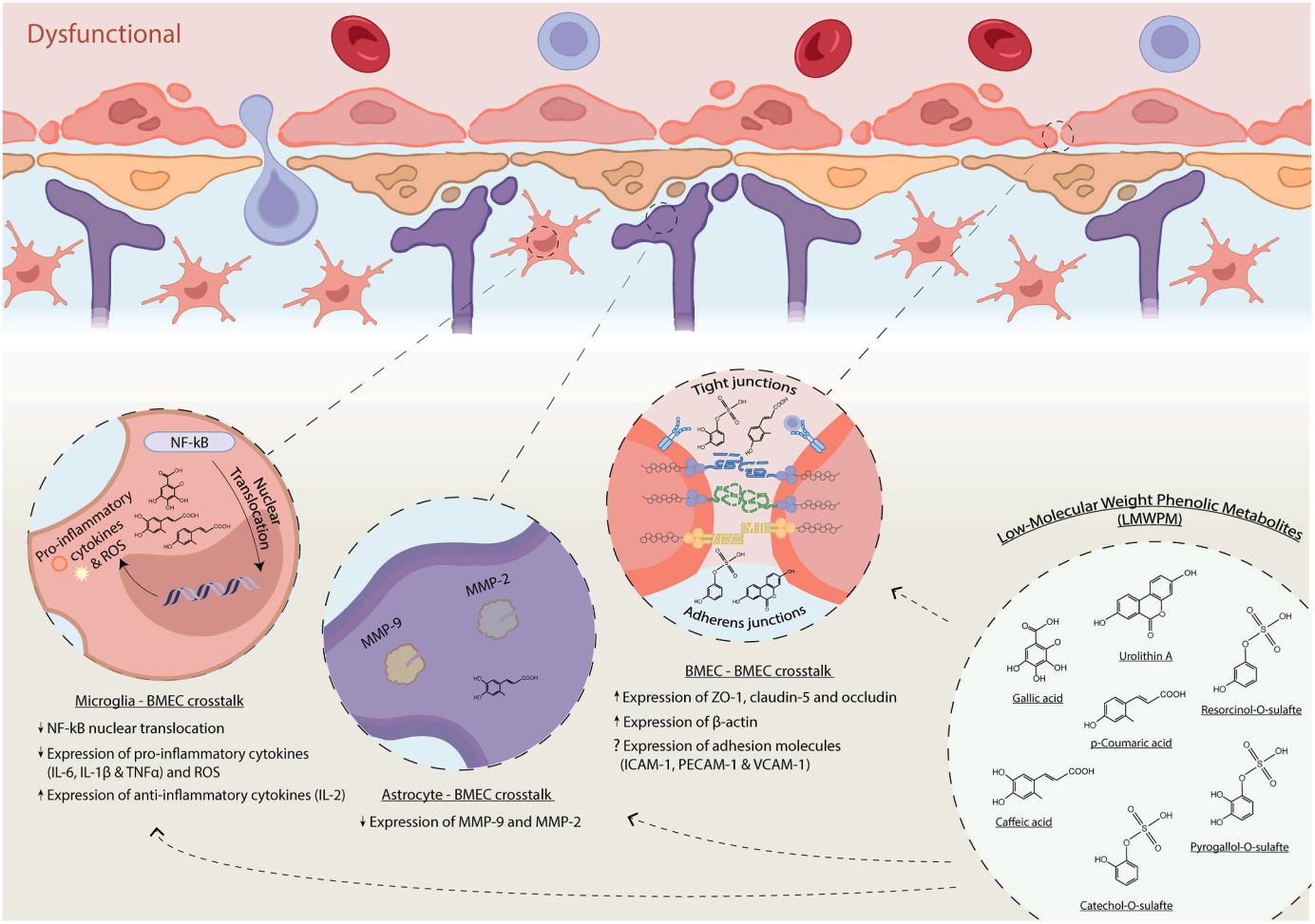

Fig. 2. Low-molecular-weight (LMW) phenolic metabolites reported beneficial effects at the blood-brain barrier (BBB) level in a disease scenario. Highlights on microglia-brain microvascular endothelial cells (BMEC) crosstalk, astrocyte-BMEC crosstalk, and BMEC-BMEC crosstalk potential of different LMW phenolic metabolites. Abbreviations: nuclear factor-κB (NF-κB), interleukin (IL), tumor necrosis factor (TNFα), reactive oxygen species (ROS), metallopeptidase (MMP), zonula occludens 1 (ZO-1), intercellular adhesion molecule 1 (ICAM-1), platelet endothelial cell adhesion molecule (PECAM-1), vascular cell adhesion molecule-1 (VCAM-1).

due to the intake and/or injection of benzoic acids, such as 4-hydroxy-3-methoxybenzoic acid (vanillic acid), 3,4-dihydroxybenzoic acid (protocatechuic acid) and 3,4,5-trihydroxybenzoic acid (gallic acid) (Carregosa et al., 2021). The same improvements have already been described with some cinnamic acids, including 4-hydroxy-3-methoxycinnamic acid (ferulic acid) and 3,4-dihydroxycinnamic acid (caffeic acid) (Carregosa et al., 2021). Also in zebrafish, few data to date reported protochatechuic acid and gallic acid improvements in fish locomotion, lipid peroxidation and oxidative stress levels restoration, as well as dopaminergic neuronal loss recovery in NDDs models (Carregosa et al., 2021). Additionally, gallic acid, ferulic acid, and caffeic acid have also been tested in Drosophila models of NDDs, with improvements in fly lifespan and locomotor activity (Carregosa et al., 2021). It is therefore clear the pleiotropic potential of these metabolites against a multiplicity of events as the ones that characterize complex and multifactorial disorders as NDDs, both *in vitro* and *in vivo*. Nevertheless, the potential of several other LMW phenolic metabolites potential against NDDs, either isolated or in combination, and taking into account their physiologically relevant circulating concentrations and pharmacokinetics is still largely underexplored and vastly unknown.

3.3. LMW phenolic metabolites attenuating BBB dysfunction

Despite the starting compiling evidence on LMW phenolic metabolites BBB transport (Carecho et al., 2021) and subsequent effects towards protein aggregation (Carregosa et al., 2021; Liu et al., 2014b), oxidative stress (Figueira et al., 2017b), and even (neuro)inflammation (Carregosa et al., 2020), the data evidencing their potential at BBB level is much rather scarce.

Regarding dietary (poly)phenols effects at BBB level, Li and colleagues (Li et al., 2012) have shown that LPS-induced inflammatory cytokine production in hCMECs could be attenuated by (−)-epigallocatechin gallate, a major (poly)phenol from green tea, via MCP-1/CCL2, VCAM-1, and ICAM-1 expression inhibition (Li et al., 2012). Additionally, salvianolic acid A (SAA), a water-soluble component derived from the root of *Salvia Miltiorrhiza Bge*, is thought to protect the BBB through (MMP-9) inhibition. Indeed, Wen et al. have demonstrated this compound's ability to attenuate BBB injury by downregulating the levels of MMP-9 and upregulating the levels of tissue inhibitor of metalloproteinase 1 (TIMP-1) (Zhang et al., 2018b). Moreover, SAA was able to significantly prevent MMP-9-induced degradation of ZO-1, claudin-5 and occludin proteins, crucial to BBB integrity, as well as preventing cerebral NF-κB p65 activation and reducing inflammatory response (Zhang et al., 2018b).

Nonetheless, further investigation is needed when it comes to LMW phenolic metabolites at the BBB level, not only at the permeability capacity of these metabolites (Carecho et al., 2021) but also at their immunomodulatory nature regarding BBB cells' crosstalk mechanisms. Fortunately, some evidence has already begun to demonstrate the potential of these metabolites at BBB level, both *in vitro* (Table 1) and *in vivo* (Table 2).

Figueira et al. studied the effects of pre-incubating an HBMEC line with LMW phenolic metabolites, such as benzene-1,2-diol-3-sulfate/benzene-1,3-diol-2-sulfate (pyrogallol-*O*-sulfate) isomer or phenol-2-sulfate (catechol-*O*-sulfate), at physiologically relevant concentrations, followed by treatment with a pro-oxidant agent - hydrogen peroxide (Figueira et al., 2017b). Besides evidencing their *in vitro* BBB transport (circa 10%), the results showed an improvement in cell viability (Figueira et al., 2017b), highlighting the protective effect of these compounds in physiological concentrations and their potential in a preventive nutritional scenario at BBB level, even if not highly BBB permeant. More recently, and using the same cell line and range of concentrations, pyrogallol-*O*-sulfate isomer emerged as the most potent LMW phenolic metabolite tested regarding BBB properties improvement, increasing β-catenin cell membrane expression and reducing ZO-1 membrane gaps, which was true after solely 2 h of exposure (Carecho et al., 2024). What can be the preventive potential of these LMW phenolic metabolites at BBB level against inflammation, either in terms

Table 1
In vitro benefits of LMW phenolic metabolites on BBB impairment and dysfunction.

	LMW phenolic metabolites	Experimental design	Model system	BBB-related outputs	Reference
Benzene diols and triols	Phenol-2-sulfate (Cathecol-*O*-sulfate)	24 h pre-incubation (5 μM)	HBMEC line	↑ cell viability after 300 μM H_2O_2 for 24h	Figueira et al. (Carecho et al., 2024)
	Benzene-1,2-diol-3-sulfate/ benzene-1,3-diol-2-sulfate (Pyrogallol-*O*-sulfate)	2h incubation (5 μM)	HBMEC line	↓ number of ZO-1 gaps per cell; ↑ membrane β-catenin; ↑ Cav1-positive vesicles; ↓ ASCT1/SLC1A4, CAT1/SLC7A1, OATP1A2/SLCO1A2, SNAT2/SLC38A2 mRNA; ↓ ASCT1, CAT1 and OATP1A2 immunoreactivity	Carecho et al. (Le Sayec et al., 2023) Figueira et al. (Carecho et al., 2024)
		24 h pre-incubation (5 μM)		↑ cell viability after 300 μM H2O2 for 24h	
	Benzene-1,3-diol-6-sulfate (Phloroglucinol-*O*-sulfate)	2h incubation (5 μM)	HBMEC line	↓ number of ZO-1 gaps per cell; ↑ β-catenin; ↑ Cav1 protein expression	Carecho et al. (Le Sayec et al., 2023)
	Phenol-3-Sulfate (Resorcinol-*O*-sulfate)	2h incubation (5 μM)	HBMEC line	↓number of ZO-1 gaps per cell; ↑β-catenin; ↑Cav1 protein expression	Carecho et al. (Le Sayec et al., 2023)
Benzoic acids	3,4-dihydroxybenzoic acid (Protocatechuic acid)	4h incubation (10 and 100 μM)	HBMEC line	β-catenin expression like the control with well-defined location at the membrane level	Gallardo-Fernandez et al. (Gallardo-Fernandez et al., 2024)
	3,4,5-trihydroxybenzoic acid (Gallic acid)	48h incubation (0.3–10 μM)	Primary rat BMEC, glial cells and pericytes (co-culture)	β-catenin and claudin-5 similar to control; ↓ NF-κB nuclear translocation (after 10 ng/ml TNF-α and 10 ng/ml IL-1β)	Ardid-Ruiz et al. (Yang et al., 2022) Taïlé et al. (Taïlé et al., 2021)
		24h incubation (10 μM)	bEnd3	↓ ROS; ↓ IL-6; ↑ MnSOD activity; counteracts up-regulation of the expression of the gene encoding Nox4 (after hyperglycemia - 33 mM glucose)	
Cinnamic acids	3′-4′-dihydroxycinnamic acid (Caffeic acid)	24h incubation (10 μM)	bEnd3	↓ MMP-2 activity; counteracts HC induced ↓ of ZO-1, ZO-2, occludin and claudin-5	Taïlé et al. (Taïlé et al., 2021)
Others	2-(3,4-Dihydroxyphenyl) ethanol (Tyrosol)	4h incubation (1, 10 and 100 μM)	HBMEC line	β-catenin expression like the control with well-defined location at the membrane level; ↓ BBB permeability (at 1 and 10 μM)	Gallardo-Fernandez et al. (Gallardo-Fernandez et al., 2024)

Table 2
In vivo benefits of LMW phenolic metabolites on BBB impairment and dysfunction.

	LMW phenolic metabolites	Experimental design	Model system	BBB-related outputs	Reference
Benzoic acids	3,8-Dihydroxy-urolithin (Urolithin A)	Intraperitoneal injection (2.5 mg/kg)	Traumatic brain injury (TBI) - Male C57BL/6J mice	↓ TBI increase in brain-water content ↑ ZO-1, occludin and β-actin; ↓ cleaved caspase-3 ↑ Bcl-2; ↑ LC3-II and ↓ p62	Gong et al. (Le Sayec et al., 2023)
Cinnamic acids	3′-4′-dihydroxycinnamic acid (Caffeic acid)	Oral gavage (35 mg/kg/day), 3 days per week for 12 weeks	High fat diet (HFD) - Ten-week-old C57BL/6	↑ ZO-1; ↓ VE-cadherin; ↓ TNF-α, IL-6, IL-1β and MCP-1; ↓ brain infarct and hemorrhagic transformation; ↓ total SOD activity and Nrf2 overproduction	Arcambal et al. (Liu et al., 2014b)
		Supplied in the diet at 0.5, 1 or 2%, either pre-intake or post-intake for 4 weeks	MPTP treated mice	↓IL-1β, IL-6, TNF-α, IL-4 and IL-10; ↓ NO, PGE2 and NOS and COX-2 activity; ↓ iNOS, nNOS, COX-2 and GFAP expression	Tsai et al. (Tsai et al., 2011)
		Oral gavage (30 mg/kg) prior to LPS (1.5 mg/kg)	Male Swiss Albino mice (8–10 weeks)	↓ LPS-induced serum TNF-α and IL-6; ↓ MDA and ↑ GSH	Basu Mallik et al. (Basu Mallik et al., 2016)
	4′-Hydroxycinnamic acid (*p*-Coumaric acid)	Oral gavage (100 mg/kg) once daily for 4 consecutive days	Hypoxic conditions (9.5% O2) for 24h - Institute of Cancer Research (ICR) male mice	↓ Brain water content ↓ BBB permeability ↓ Evans blue extravasation ↑ Occludin; ↓IL-2 and ↑ IL-10; ↑ SOD and CAT activity; ↑ GSH	Li et al. (Carecho et al., 2024)
	3-(3-methoxy-4-hydroxyphenyl) propenoic acid (Ferulic acid)	Oral gavage for 6 months	Transgenic PSAPP mouse model of cerebral amyloidosis (bearing mutant human APP and presenilin-1 transgenes)	↓ brain mRNA expression of TNF-α and IL-1β; ↓ Sod1, catalase, and Gpx1 expression	Mori et al. (Mori et al., 2013)
		Oral gavage (20, 40 or 80 mg/kg), once a day for 4 weeks	Male ICR mice (6 weeks old) exposed to chronic unpredictable mild stress (CUMS)	↓ IL-1β expression in the serum; ↓ NLRP3 and caspase-1 expression	Liu et al. (Liu et al., 2017)

of junctional proteins improvement, cytoskeleton, or even towards adhesion and pro-inflammatory response is for the time being unknown. Importantly, phloroglucinol aglycone (*i.e.,* without the sulfate group) has already been shown to decrease glial activation and pro-inflammatory cytokine (TNF-α and IL-6) expression and release, ameliorating cognitive impairments and Aβ burden in 5xFAD mice (Yang et al., 2018), further corroborating our hypothesis that phloroglucinol and or its derived metabolites can present powerful anti-inflammatory bioactivity in the scope of NDDs.

Regarding LMW phenolic metabolites modulation of TJ and AJ proteins *in vitro* (Table 1), caffeic acid has already been reported to counteract the decrease of ZO-1, ZO-2, occludin and claudin-5, induced by hyperglycemia in murine bEnd3 cerebral endothelial cells (Taïlé et al., 2021). Another study using a co-culture model of primary rat BMEC, glial cells and pericytes, reported that solely 1 μM of gallic acid was able to maintain β-catenin and claudin-5 levels similar to control, when faced with TNF-α and IL-1β inflammatory insult (Schroeter et al., 2006). Indeed, (−)-epicatechin-derivatives are believed to mediate the beneficial effects of flavanol-rich cocoa on the endothelium and vascular function by increasing nitric oxide bioavailability and reducing vasoconstrictors, such as endothelin-1 (Schroeter et al., 2006).

As for *in vivo* models of NDDs where compromised BBB integrity and function occurs, some LMW phenolic metabolites have already shown some improvements in BBB permeability/integrity related outcomes (Table 2) (Yang et al., 2022; Kumar et al., 2021; Li et al., 2019). Although understudied, some LMW phenolic metabolites have been reported to exert a positive regulation in TJ proteins, like Gong et al. that presented the effect of urolithin A, a metabolite that results from gut bacteria transformations of dietary (poly)phenols, in alleviating BBB disruption by predominantly upregulating ZO-1, occludin, and β-actin (Gong et al., 2022). This is an example of the intrinsic potential that these compounds carry, and although further testing is necessary to support the growing evidence linking LMW phenolic metabolites and TJ modulation. Regarding AJs expression alterations, and surprisingly, Arcambal et al. reported lower levels of VE-cadherin in BBB upon intraperitoneal injection with caffeic acid (50 mg/kg body weight) on C57BL/6 mice exposed to hyperglycemia, though a concomitant up-regulation of ZO-1 levels was also observed (Arcambal et al., 2020).

Regarding clinical data in humans, the observations on the effects of LMW metabolites and even dietary (poly)phenols at BBB level are even scarcer. Compiling evidence demonstrates that higher dietary intake of (poly)phenol-rich foods is associated with better vascular and cognitive performance and a lower risk of NDDs development, mainly associated with their anti-inflammatory, antioxidant, antiplatelet, and vasodilatory potential. (Poly)phenolic compounds have been shown to be capable of lowering blood pressure and improving cerebral blood flow (Haskell-Ramsay et al., 2018; Fraga et al., 2011; Rendeiro et al., 2015; Cheng et al., 2017). Blueberries feeding for 12 weeks improved cerebral blood flow on both parietal and occipital lobes in healthy older adults (Bowtell et al., 2017). Clinical trials have been conducted to elucidate blueberry supplementation and its mechanism of action on brain health, namely towards cognitive performance and by enhancing vascular efficiency in the elderly (NCT02446314, 2015; NCT04049162, 2019). Moreover, a wild blueberry extract intervention in healthy older adults

reduced both systolic and diastolic blood pressure and attenuated cognitive decline, particularly during the circadian rhythm-driven postprandial dip, being aligned with the temporal increase in plasma blueberry (poly)phenol metabolites (Cheng et al., 2024). Not only berries but also acute cocoa supplementation enhanced the visual performance of young adults due to increased blood flow to the retina and brain (Field et al., 2011) and high isoflavone intake was associated with a reduced risk of myocardial and cerebral infarction in Japanese women (Kokubo et al., 2007). What are the exact mechanisms governing bioavailable (poly)phenols or LMW metabolites' effects in BBB homeostasis or even in the prevention of BBB leakage resulting from stroke, TBI or NDDs progression is for the time being still unknown.

In the scope of putative effects associated with LMW metabolites, what has been shown so far is that grape and blueberry extract supplementation can improve age-related episodic memory decline in healthy elderly individuals with a lower level of memory performance, where urinary concentrations of specific flavan-3-ols metabolites were associated with those memory improvements (Bensalem et al., 2019). Moreover, a meta-analysis from randomized clinical trials suggested that chlorogenic acid intake can cause statistically significant reductions in systolic and diastolic blood pressures (Onakpoya et al., 2015). And four hydroxybenzenes - resorcinol, pyrogallol, phloroglucinol, and 4-methylcatechol - despite having been tested at supraphysiological concentrations (*i.e.*, 240 μM), displayed a relevant antiplatelet activity in human blood, where pyrogallol had a comparable effect to acetylsalicylic acid and 4-methylcatechol emerged as the most efficient being about 10 × times more active than clinically used acetylsalicylic acid (Applová et al., 2019). All this evidence gives hope for the pursuit of LMW (poly)phenol metabolites in clinical settings at BBB level.

Altogether, evidence supports that LMW phenolic metabolites can constitute promising and possibly revolutionary compounds against NDDs, given their significant concentrations in circulation, permeating capacity of the BBB, and modulation AJ and TJ expression levels, among others (Fig. 2). Despite all of that, future studies need to fully understand if the presumed neuroprotective nature of these compounds directly modulates inflammation at BBB level, or if it is even viable in the crosstalk environment should be addressed. Efforts should be made also by replicating different nutritional paradigms that diets rich in (poly) phenols can have at BBB level and identifying the LMW phenolic metabolites responsible for the different molecular events. Only then we will be able to dissect to its full extent, the intrinsic mechanism in NDD progression and act accordingly, so that these pathologies become inconsequential for future generations.

4. Future considerations in LMW phenolic metabolites research

It is important to highlight that *in vivo*, these LMW metabolites do not exist in isolation but rather in a complex biological environment where pharmacokinetics and synergistic interactions with other molecules can significantly influence their ultimate biological effects. And, for the time being, these LMW phenolic metabolites have been experimentally tested alone. What will be the potential of this LMW phenolic metabolite when combined, mimicking a real nutritional apport as it occurs when a (poly) phenol-rich meal? Will the potential of each LMW phenolic metabolite be additive or antagonistic? And more, since the kinetics of each LMW phenolic metabolites varies over time and also according to gut inter-individual variability, what will be the effects of different LMW phenolic metabolite "physiological mixtures" and at different timepoints? The answers to these questions are yet to be clear.

Moreover, most of the data published so far on the cellular and molecular mechanisms of (poly)phenols and LMW phenolic metabolites against NDDs has been performed taking advantage of simplistic cellular systems, where intra-/intercellular communication mechanisms are not contemplated. There is a critical necessity in conducting LMW phenolic metabolites mechanistic studies in more translational, physiological, and relevant human cellular systems that consider the brain complexity and bypass the need for extensive animal experimentation. Indeed, the 3D architecture of spheroids/organoids system more closely recapitulates the complexity of *in vivo* settings, being particularly important when considering the brain microenvironment (D'Antoni et al., 2023), though still underexplored in (poly)phenols research. Additionally, microphysiological systems (MPS) have emerged as powerful tools to study neurodegenerative diseases, better emulating *in vivo* organ architecture and mimicking the physiological aspects of function/pathophysiological condition, representing a very promising roadmap for drug discovery and clinical testing, bypassing animal experimentation (Kavand et al., 2022; Fda; Miccoli et al., 2018). The United States Food and Drug Administration defines MPS as "a microphysiological system which uses microscale cell culture platform for *in vitro* modeling of functional features of a specific tissue or organ of human or animal origin by exposing cells to a microenvironment that mimics the physiological aspects important for their function or pathophysiological condition" (FDA). In fact, MPS design may provide and support cultured cells with physical (*e.g.*, temperature, pH and oxygen), biochemical, electrical, mechanical (*e.g.*, flow or stretch), structural, morphological conditions and recapitulate specific properties that define a healthy or diseased organ/tissue function. Probably due to enormous breakthrough in the past years, MPS is an inclusive term, encompassing body-on-chips and organ-on-chips. Organ-on-chips (OoC) are a subset of MPS with microfluidic cell cultures especially suitable to recapitulate tissue-tissue interfaces, highly versatile platforms that enable miniaturization and advanced control, where the reduced sizes come with considerable benefits, as diminished reagent costs and increased analysis accuracy (Staicu et al., 2021). Notwithstanding, more advanced OoC can be generated with human induced pluripotent stem cells (iPSC) to allow the establishment of patient- and disease-tailored systems, where different cell subtypes may be obtained from the same donor (Shi et al., 2017). Such OoC will be extremely advantageous to mimic the real nutritional effects of particular metabolites (*e.g.*, LMW phenolic metabolites) found in the bloodstream, which fluctuate in their concentrations over time. Despite the clear advantages, such devices were never used before to study (poly)phenols nor LMW phenolic metabolites effects. We strongly believe that, to push forward the knowledge on LMW phenolic metabolite cellular and molecular effects towards NDDs, the future of research will need to pass by the use of such state-of-art advanced and more complex cellular systems, profiting from more representative human cell sources, and recapitulating the brain microenvironment complexity.

5. Conclusion

The era of focusing on the real nutritional value and the molecular mechanisms behind LMW phenolic metabolites in the prevention of NDDs is just emerging. Although the study of such metabolites at BBB level and beyond is still in its infancy, it is a rapidly growing field with great potential. The accumulating evidence suggests that these metabolites are pleiotropic compounds, offering promising therapeutic avenues for NDDs, either through preventive nutritional approaches or pharmaceutical interventions. Even of not highly BBB permeant, LMW phenolic metabolites are strong bioavailable candidates to boost BBB properties at the same range of concentrations as they are found in the bloodstream. This review just lift the veil over the few data available to date regarding LMW phenolic metabolites effects modulating NDDs and, in particular, at BBB level. We foresee that, in the future, besides further compiling animal studies, also more complex *in vitro* cell systems as co-culture systems, profiting from the advent of iPSC, organoids and MPS/OoC will be employed to help researchers to fully disclose the true mechanisms in more physiological relevant settings.

CRediT authorship contribution statement

Daniela Marques: Writing – review & editing, Writing – original draft, Investigation. **Diogo Moura-Louro:** Writing – review & editing,

Writing – original draft, Investigation. **Inês P. Silva:** Writing – review & editing, Writing – original draft, Investigation. **Sara Matos:** Writing – review & editing, Writing – original draft, Investigation. **Cláudia Nunes dos Santos:** Writing – review & editing, Validation, Funding acquisition, Data curation. **Inês Figueira:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

Hereby we declare that this is an original publication, that was not published or submitted elsewhere.

The authors alone are responsible for the content enclosed in the manuscript.

All the authors declare to have no conflict of interest.

Data availability

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