


Flavins and Flavoproteins in the Neuroimmune Landscape of Stress Sensitization and Major Depressive Disorder

Matt Scott Schrier ¹, Maria Igorevna Smirnova ²⁻⁴, Daniel Paul Nemeth ¹,
Richard Carlton Deth ⁵, Ning Quan ^{1,3}

¹Department of Biomedical Science, Charles E. Schmidt College of Medicine, Florida Atlantic University, Jupiter, FL, USA; ²The International Max Planck Research School (IMPRS) for Synapses and Circuits, Jupiter, FL, USA; ³Stiles-Nicholson Brain Institute, Florida Atlantic University, Jupiter, FL, USA; ⁴Department of Biological Sciences, Charles E. Schmidt College of Science, Florida Atlantic University, Jupiter, FL, USA; ⁵Department of Pharmaceutical Sciences, Barry and Judy Silverman College of Pharmacy, Nova Southeastern University, Ft. Lauderdale, FL, USA

Correspondence: Matt Scott Schrier, Florida Atlantic University, Building: MC-17, Room: 229E, 5353 Parkside Drive, Jupiter, FL, 33458, USA, Email mschrier@health.fau.edu

Abstract: Major Depressive Disorder (MDD) is a common and severe neuropsychiatric condition resulting in irregular alterations in affect, mood, and cognition. Besides the well-studied neurotransmission-related etiologies of MDD, several biological systems and phenomena, such as the hypothalamic-pituitary-adrenal (HPA) axis, reactive oxygen species (ROS) production, and cytokine signaling, have been implicated as being altered and contributing to depressive symptoms. However, the manner in which these factors interact with each other to induce their effects on MDD development has been less clear, but is beginning to be understood. Flavins are potent biomolecules that regulate many redox activities, including ROS generation and energy production. Studies have found that circulating flavin levels are modulated during stress and MDD. Flavins are also known for their importance in immune responses. This review offers a unique perspective that considers the redox-active cofactors, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), as vital substrates for linking MDD-related maladaptive processes together, by permitting stress-induced enhancement of microglial interleukin-1 beta (IL-1 β) signaling.

Keywords: cofactor, cytokines, IL-1 β , microglia, neuroinflammation, redox

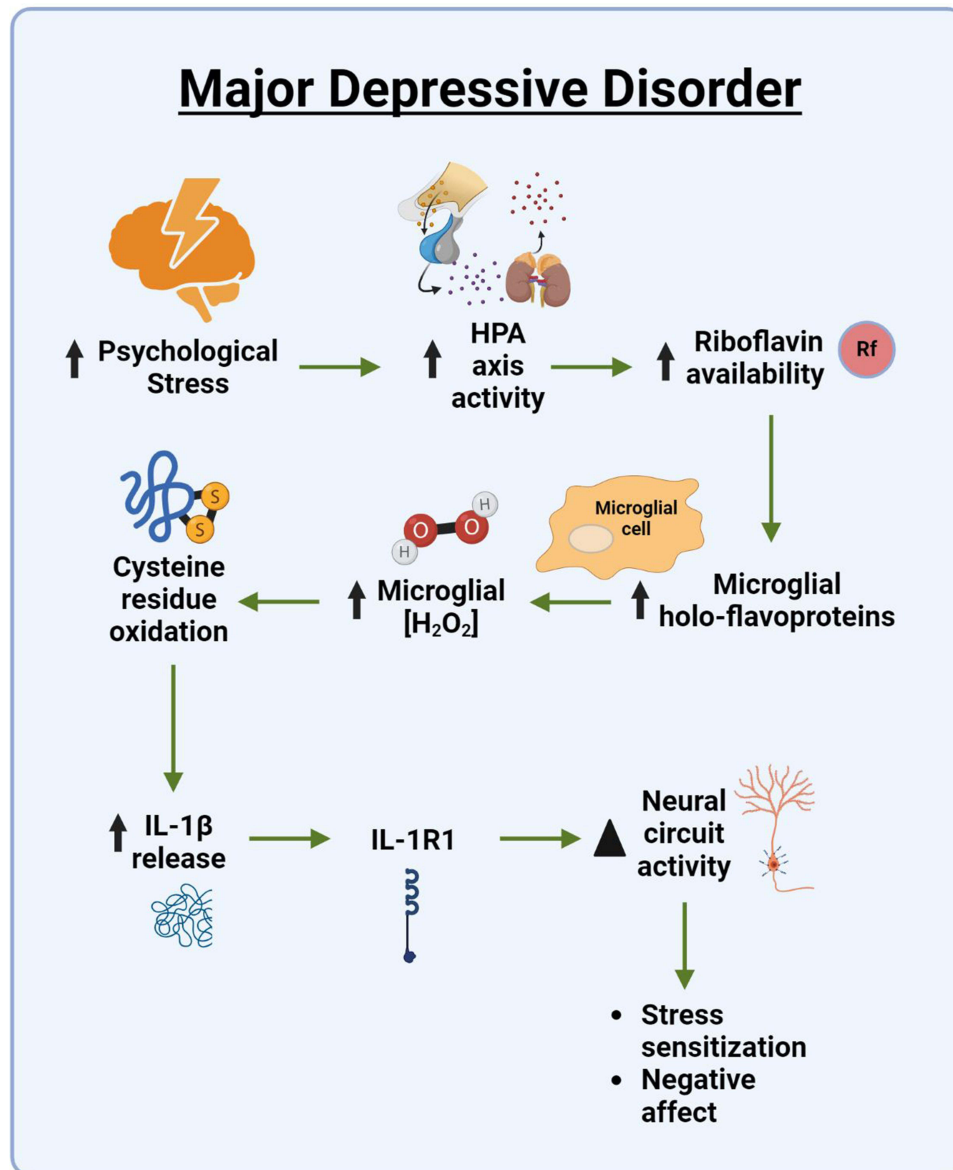
Introduction

General introduction

Major depressive disorder (MDD) is linked with an increased risk of suicide, which claimed over 45,000 lives in the United States in 2020.¹ MDD is also reported to increase the risk of other neurological disorders, including Alzheimer's disease,^{2,3} as well as increasing overall mortality risk.⁴ For decades, MDD treatment has focused on antidepressant drugs that increase levels of extracellular monoamines, especially serotonin, in the brain. These drugs, such as selective serotonin reuptake inhibitors (SSRIs), are usually helpful for those with MDD⁵ and may decrease suicide risk.⁶ However, the effectiveness of current antidepressants is limited, and a substantial percentage of individuals with MDD do not obtain adequate relief from treatment.⁷

Involvement of the immune system in depression and in antidepressant resistance has gained increased attention in recent years. The immune system conveys messages to other cell types in the body through many mediators, such as cytokines. Some of the first evidence for a cytokine-mediated etiology of depressive symptoms came to light when treatment of healthy controls or cancer patients without a history of depression with Interleukin-2 or Interferon-alpha led to depressive symptoms.^{8,9} These data suggested that immune mediators, specifically cytokines, are important for the generation of depressive symptoms. Interleukin-1 beta (IL-1 β) is one of the primary cytokines produced and released in response to stressors.^{10,11} Interestingly, elevated serum IL-1 β levels have been found in some patients with MDD¹² and

Graphical Abstract



pre-clinical studies support a causal role for IL-1 β in the induction of depression-like phenotypes in rodents.^{13–15} Basic knowledge of the physiological activities of IL-1 β is key for understanding its implications for MDD development. IL-1 β transmits pro-inflammatory messages via binding to and activating interleukin-1 receptor type I (IL-1R1),¹⁶ expressed on the surface of immune, endothelial, and other cell types. In leukocytes, IL-1R1 activation increases transcription and subsequent translation of a variety of cytokines, chemokines, and enzymes. In this manner, IL-1 β /IL-1R1 coordinates the immune response to infection. In addition, IL-1R1 is expressed by a variety of peripheral tissues¹⁷ and cell types in the brain,^{18,19} including neurons. Neuronal IL-1R1 has been thought to regulate affect, learning, memory, and certain behaviors.²⁰

The presence of IL-1R1 throughout the body and brain enables IL-1 β to influence neuronal activity by interacting with several systems that also have neuromodulatory effects. For example, IL-1 β /IL-1R1 signaling in dorsal raphe nucleus neurons has long been associated with regulation of the brain serotonin system.^{21–24} IL-1 β /IL-1R1 signaling also

engages in important cross-talk with the hypothalamic-pituitary-adrenal (HPA) axis, stimulates formation of reactive oxygen and nitrogen species (ROS and RNS, respectively), and decreases mitochondrial function. Most of the systems that IL-1 β modifies, can also feedback to regulate IL-1 β . These relationships form a coordinated web of interacting systems, which endow organisms with the ability to respond to a multitude of stimuli while maintaining homeostasis under either normal or adverse conditions. However, deficits in metabolic and nutritional factors can lead to dysfunction in these sophisticated networks, contributing to MDD.

Vitamins and minerals are critical for the function of many proteins and enzymes required by animal species for survival. Riboflavin (Rf), more commonly known as Vitamin B2, is an inactive precursor to the enzymatic cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) (Figure 1A) that support the activity of at least 90 proteins (flavoproteins) in humans.²⁵ Collectively, Rf, FMN, and FAD are called flavins. While Rf deficiency is rare in developed countries, suboptimal flavin status or functional deficiency can occur as a result of inadequate nutrition, age, and various health states (eg, pregnancy, alcohol abuse, digestive disorders, thyroid diseases, etc).²⁶ Flavins are useful for redox reactions, (as will be reviewed below), but they can also participate in other types of chemical reactions. Thus, flavoproteins are posited to serve pleiotropic roles. Flavins and flavoproteins are necessary for mitochondrial energy production,^{27–29} immune system function,³⁰ hormone synthesis and responsiveness,³¹ ROS and RNS production and detoxification, tryptophan utilization,^{32–34} and neurotransmitter turnover^{35,36} - among many other functions. These functions highlight the utmost importance of flavoproteins in the interaction between the immune and neural systems. Participation of flavoproteins in these broad domains suggests that alterations in flavin availability can influence neuroimmune, neuroendocrine, and metabolic activities, and consequently affect both the acute response to and long-term effects of pro-inflammatory and psychological stressors. Flavin deficiency may cause changes in affective states³⁷ and decrease the bioenergetic capacity for physical activity.^{37–42} Notably, decreased flavin levels were found in a cohort of patients with MDD in remission.⁴³ Despite the link between decreased flavins and MDD, based upon the foregoing, we hypothesize that misappropriation of flavins by cells of the immune system can also have adverse mental health consequences that manifest due to increased neuroimmune signaling.

Importantly, stress and inflammation modify flavin and flavoprotein status. Stress- and inflammation-induced neuroendocrine and immune programs alter extracellular flavin availability^{44–46} and intracellular allocation, as well as the level and activity of specific flavoproteins.^{47,48} Indeed, under such deviations from homeostasis, with respect to cell type-specific adaptations, flavin utilization, and flavoprotein activity may shift from normal metabolic functions, mitochondrial oxidative phosphorylation,^{49–51} and reduction-oxidation (redox) signaling, towards enhanced ROS/RNS generation^{52,53} through upregulation of specific flavoproteins or activation of mitochondrial reverse electron transport.⁵⁴ This shift could permit increased IL-1 β synthesis through redox-dependent mechanisms, which bolsters the immune response to a broad spectrum of potential threats. It may also support a cascade of IL-1 β -dependent processes in the central nervous system (CNS) that orchestrate a transition towards depressive psychiatric illness. Here we discuss how stress- and inflammation-induced changes in systemic flavin availability can support redox alterations that promote increased IL-1 β synthesis in the brain, leading to depression symptoms. To assess the feasibility that flavins could have a role in MDD, we screened the PubMed database for articles related to flavins and stress, infection, MDD, depression, ROS, inflammation, or IL-1 β . Our query was inclusive of research articles published during any year. Additionally, while it has received relatively little attention, studies have suggested that antidepressant medications that elevate synaptic serotonin and other monoamines have significant interactions with flavins and flavoproteins.^{46,55,56} These interactions may account for some of their pharmacological benefits for MDD symptoms, and also suggest that Rf availability could be a determinant of treatment efficacy. We describe the literature on this subject, as well as other translational implications of flavins for MDD patients.

Introduction to Flavins and Cellular Flavin Acquisition

Since many excellent reviews on flavins and flavoproteins and their importance in human health have been published, this general introduction will be limited to information necessary for understanding the current focus, and we refer the reader to Powers (2003),⁵⁷ Lienhart et al (2013),²⁵ and Mosegaard et al (2020)⁵⁸ for additional background knowledge. Carbon-based life would not be possible without cofactors to facilitate enzymatic catalysis of thermodynamically-unfavorable chemical

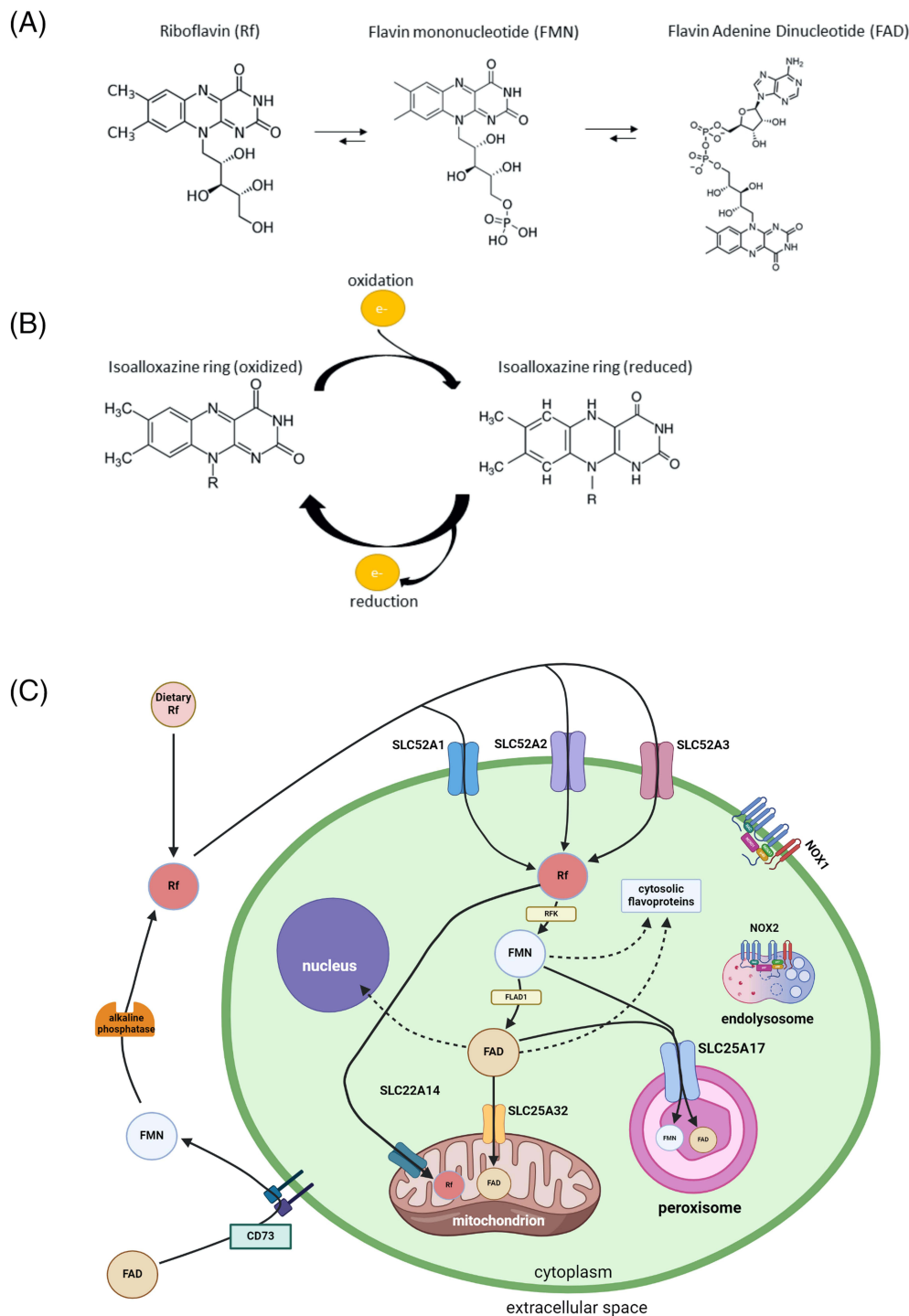


Figure 1 (A) Interconversion of flavins. Riboflavin (Rf) is converted enzymatically to Flavin mononucleotide (FMN) by its phosphorylation. Adenosine monophosphate is bound to the phosphate group of FMN to form Flavin adenine dinucleotide (FAD). (B) Basis of Flavin utility. This is a simplified schematic depicting the isoalloxazine ring participating in a redox reaction. The isoalloxazine ring can exist in three states. Nitrogen or a carbon atom of the isoalloxazine ring can accept electrons from a substrate that is then oxidized. One or two electrons may be accepted and donated. A more detailed review of the biochemistry of isoalloxazine rings can be found in Zemleni et al.¹⁷⁰ (C) Cellular distribution and incorporation of flavins. Extracellular Rf is derived from diet, but it can also be regenerated from FMN or FAD by enzymatic catabolism by alkaline phosphatase and Cluster of Differentiation 73 (CD73), respectively. Cellular Rf uptake is mediated by Solute Carrier Family (SLC) 52 transporters 1, 2 and 3. Riboflavin kinase (RFK) is responsible for forming FMN from Rf. FAD synthetase (FLAD1) converts FMN to FAD. FMN and FAD are incorporated into cytosolic flavoproteins. Rf, FMN, and FAD can be distributed to subcellular compartments by a few identified transporters. SLC25A32 and SLC22A14, respectively, transport FAD and Rf into mitochondria. SLC22A14 is mostly present in a single cell type.⁶⁹ It is thought that FAD in mitochondria may be catabolized to FMN to be used for complex I.²⁸ Peroxisomal FMN and FAD are imported via SLC25A17. Flavins comprise many reactive oxygen species-producing enzymes in each subcellular compartment. NADPH oxidase 2 (NOX2) is localized to endolysosomes, and NOX1 is localized to the plasma membrane. Flavins and flavoproteins are also found in the nucleus. Created in BioRender. Nemeth, D (2024) BioRender.com/z17n701.

reactions. While nature has a variety of cofactors, the most versatile examples support multiple reaction mechanisms and participate in the activities of numerous enzymes, reflecting the evolution of proteins which exploit the capabilities of these cofactors. Flavins have an isoalloxazine ring system that is capable of efficiently accepting and transferring one or two electrons (and protons) via nitrogen or carbon atoms in the ring, making flavins very useful for redox-related biochemical reactions (Figure 1B). The precursor flavin, Rf, is converted to FMN and FAD (Figure 1A and B) and while FMN is used by only a handful of enzymes, FAD is used by dozens of proteins that reside in the cytoplasm and within several organelles, most notably in mitochondria. However, a few enzymes require both cofactors.

As depicted in Figure 1C, Rf is transported into cells by one of three transporters in humans: SLC52A1,⁵⁹ SLC52A2,^{60,61} and SLC52A3.^{62,63} At physiological concentrations, FMN and FAD cannot be taken up directly and circulating FMN and FAD must be converted back to Rf first. CD73 hydrolyzes FAD and alkaline phosphatase further degrades FMN to Rf.⁶⁴ Once in cells, Rf is phosphorylated to FMN by Rf kinase (RFK)⁶⁵ and FMN is subsequently converted to FAD by FAD synthetase (FLAD1), which is present in mitochondria and in the nucleus, where it supports nuclear flavoproteins.⁶⁶ FMN and FAD can be imported into peroxisomes by SLC25A17, which is a transporter for several other cofactors.⁶⁷ FAD can also be transported into mitochondria by SLC25A32.⁶⁸ A recently published investigation showed that SLC25A32 knockout results in less mitochondrial flavin content in cultured cells, which was due to loss of complex I of the mitochondrial electron transport chain.²⁸ A mitochondrial Rf transporter, SLC22A14, was characterized recently⁶⁹ that could also account for mitochondrial flavin entry, but its expression was deemed to be highly tissue-specific.

The Central Role of Flavins in Redox Biochemistry

Flavins support numerous basic cellular functions and physiological activities. FAD and/or FMN are involved in fatty acid beta-oxidation, oxidative phosphorylation, Coenzyme A synthesis,⁷⁰ Vitamin B6,⁷¹ and B12 utilization,^{72–76} circadian rhythm maintenance,⁷⁷ protein folding,⁷⁸ reduced glutathione regeneration,⁷⁹ tryptophan and serotonin metabolism, hydrogen sulfide metabolism,^{80,81} thyroid hormone metabolism and response,^{31,82–84} as well as cholesterol synthesis.⁸⁵ Most importantly for the current review, we note that flavins are crucial for their role in ROS formation and redox signaling. The vast majority of human enzymes that produce superoxide or hydrogen peroxide (H₂O₂)⁸⁶ contain flavin cofactors. Superoxide spontaneously converts to H₂O₂. However, superoxide dismutase enzymes also convert superoxide to H₂O₂. H₂O₂ then oxidizes thiol groups of vulnerable protein cysteines. The activities of many proteins that have oxidizable cysteine residues are influenced by the presence of elevated concentrations of H₂O₂. Cells use ROS-generating proteins, such as NADPH oxidases, as a means to regulate the activity of other proteins by controlling their cysteine thiol group oxidation state. The cysteine oxidation in this kind of signaling, termed redox signaling, is reversible.⁸⁷ A classic example of redox signaling is the oxidation of cysteine residues on tyrosine phosphatases by H₂O₂ following stimulation of cells with trophic factors. Oxidation of tyrosine phosphatase cysteine residues inhibits phosphatase activity, which prolongs activation of receptor tyrosine kinases (RTKs) by trophic factors.⁸⁸ RTKs induce acute NADPH oxidase activity. The superoxide produced is converted to H₂O₂, which inhibits the phosphatases. The particular importance of redox signaling in initiating and sustaining inflammation will be explained in further detail in Flavins, Flavoproteins, and IL-1 β in Stress Sensitization, and MDD.

Stress, Inflammation, IL-1 β Signaling, and MDD

Psychological and chronic stressors are risk factors for and often precipitate MDD episodes.⁸⁹ The primary physiological response to a stressor is the activation of the HPA axis. Exposure to stressful events increases the release of corticotropin-releasing factor (CRH) from the paraventricular nucleus of the hypothalamus, which binds to CRH receptors to induce adrenocorticotropic hormone (ACTH) release from the anterior pituitary. ACTH binds to ACTH receptors on cells of the adrenal cortex. ACTH receptors mediate secretion of glucocorticoids. Glucocorticoids induce a range of effects that may contribute to induction of depression; as reviewed elsewhere.⁹⁰ Notably, glucocorticoids exert immunosuppressive effects through binding to the intracellular glucocorticoid receptor, which suppresses transcriptional activity of the pro-inflammatory transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B). The glucocorticoid receptor also suppresses inflammation through a recently described metabolic effect.⁹¹ However, during chronic or

severe stress, glucocorticoid-resistance may occur, which antagonizes the immunosuppressive effect of the glucocorticoid receptor on NF- κ B.⁹² One cause of glucocorticoid-resistance is downregulation of glucocorticoid receptor protein levels.⁹³

Glucocorticoid-resistance leads to increased immune activation; both of which are commonly documented occurrences in MDD subjects.⁹⁴ A correlate of immune cell reactivity is the production of cytokines and increased peripheral levels of pro-inflammatory cytokines have been detected in many MDD studies.⁹⁵ Some of those studies found IL-1 β to be increased. The inconsistency of changes in peripheral IL-1 β may be explained by the typically short duration that IL-1 β is elevated during an immune response⁹⁶ and by the inaccuracy of measuring minor changes in IL-1 β protein, which exist at extremely low (sub-picogram per 100 ug protein in the brain under basal conditions) abundance.⁹⁶ Several clinical studies also detected increased expression of inflammasome components in MDD subjects.^{97–99} These results are consistent with findings of increased inflammasome expression and/or IL-1 β production in animals following in vivo stress paradigms,^{93,100–102} selective upregulation of NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) expression in the brain of stress-susceptible but not stress-resilient mice,¹⁰³ and a requirement for NLRP3 expression for stress sensitization.¹⁰⁴ It should be noted that the involvement of IL-1 β in mediating many of the outcomes of stress requires its interaction with neuronal IL-1R1, although studies have shown that blood-brain barrier endothelial cell IL-1R1 expression is also important for stress pathology.^{100,102}

In the CNS, pro-inflammatory elevations in IL-1 β are largely due to stimulation of microglia,^{18,105,106} and microglial depletion has revealed their importance for the observed behavioral effects of stress.^{100,107} Following stress, microglial glucocorticoid-resistance occurs and causes increased microglial IL-1 β production.^{92,93} Our lab, along with collaborators, have shown that hippocampal dentate gyrus glutamatergic neuronal IL-1R1 expression is requisite for neuronal adaptations to stress, as well as accompanying cognitive deficits and behavioral responses.¹⁰⁸ Mice lacking neuronal IL-1R1 (nIL-1R-null mice) are less prone to developing social deficits and anxiety following social defeat stress. The precise downstream mechanisms of glutamatergic neuronal IL-1R1 activation that mediate stress sensitization still need to be elucidated. However, following a multi-day social defeat stress paradigm, nIL-1R1-null mice do not display increases in the neuronal activation markers Delta FosB or phospho-CREB,¹⁰⁸ implying that neuronal IL-1R1 is required to encode the stressful event.

Additional aspects of IL-1 β /IL-1R1 signaling may contribute to depression. Exposure to sufficient levels of cytokines or to toll-like receptor agonists, such as lipopolysaccharide (LPS) or polyinosinic:polycytidylic acid (Poly (I:C)) induces a set of behaviors known as sickness behaviors, which include fatigue, decreased locomotor activity, and decreased sociability, fever, and decreased food consumption. Some sickness behaviors are like behaviors seen in humans during depression and it has been suggested depressive features could arise from the same neural pathways that mediate some sickness behaviors.¹⁰⁹ Our lab showed that sickness behavior induced by intracerebroventricular injection of IL-1 β was dependent on brain endothelial cell IL-1R1 and required the expression of endothelial cyclooxygenase-2.¹⁸ In another study, peripheral administration of low-dose (0.2 mg/kg) LPS led to distinct depression-like behaviors in mice within one hour without the symptoms of a typical sickness response, but this did not occur in IL-1R1-knockout or in serotonin transporter- (SERT) knockout animals.¹⁵ It is believed that the depressive-like effects of LPS observed by Zhu et al¹⁵ were induced by increased activation of SERT by IL-1R1 expressed on serotonergic neurons. Serotonergic neuronal IL-1R1 increases SERT activity through activation of p38 α MAPK.^{24,110} Such behavioral responses to low-dose LPS did not occur in mice that had serotonergic neuron-specific genetic ablation of p38 α MAPK.¹¹¹ Other effects of increased CNS IL-1 β with relevance for depression include the ability of IL-1 β to impair hippocampal neurogenesis,¹¹² antagonize brain-derived neurotrophic factor signaling,^{113,114} promote kynurenine pathway activation,¹¹² and stimulate the HPA axis.^{115,116}

Flavins, Flavoproteins, and IL-1 β in Stress Sensitization, and MDD

Stress-Induced Changes in Flavin Levels

An important function of glucocorticoids is to increase nutrient availability to assist the body to adapt to stressors. Both psychological stress and pathogen infection,^{45,46,117} a physiological stressor, temporarily increase circulating flavin levels via mobilization from hepatic tissue. This may be due to elevated blood glucocorticoids.^{118–120} Under glucocorticoid exposure, the liver may release other B vitamins, in addition to flavins.¹¹⁹ Transient elevation of plasma B vitamins and

their mature cofactors, including FAD, was also observed in mice injected with ATP,¹²¹ which highlights the generalizability of this biological response to stressors. Brijlal et al (1996)¹¹⁷ suggested that the increase in flavin mobilization during infection may support the immune response against pathogens. FAD released by the liver during inflammation is broken down extracellularly to FMN and Rf. Consequentially, an enhanced provision of Rf becomes available to peripheral leukocytes during periods of elevated exposure to stressors. Following uptake by leukocytes, intracellular Rf is converted back to FMN by RFK and subsequently to FAD, which are incorporated into flavoproteins involved in pathogen defense (eg, inducible NOS; iNOS).

Specific Roles of Flavoproteins in Neuroinflammation and MDD

Similar to the conceptualization proposed by Brijlal et al,¹¹⁷ we postulate that during psychological stress-induced HPA axis activation, acute flavin redistribution from the liver compartment to the bloodstream functions to enhance both peripheral- and CNS immune activation, contributing to pathological immune-related processes that enhance stress susceptibility (Figure 2); these activities may include ROS synthesis, increased kynurenine pathway metabolism, and cytokine production. In support of this idea, corticosterone treatment increased plasma Rf in a rat postpartum depression model, and the length of time that rats spent immobile in the forced swim test was positively correlated with plasma FMN.¹²⁰ Corticosterone concurrently increased 3-hydroxykynurenine, the breakdown product of kynurenine produced by the flavoprotein kynurenine-3-monooxygenase (KMO). 3-hydroxykynurenine is a toxic metabolite that induces ROS production.^{122,123} The authors of a social-defeat stress study,⁴⁶ assigned C57BL/6 mice into stress-susceptible- and stress-resilient groups based upon their willingness to interact with a novel CD-1 mouse after 10 days of undergoing social-defeat stress. Metabolomic data was acquired from stress-susceptible, stress-resilient, and control mice. The authors observed that mice which were susceptible to social-defeat stress tended to have higher serum FAD Z-scores than both control mice and stress-resilient mice. Lastly, serum levels of the ROS-producing flavoprotein NADPH oxidase 1

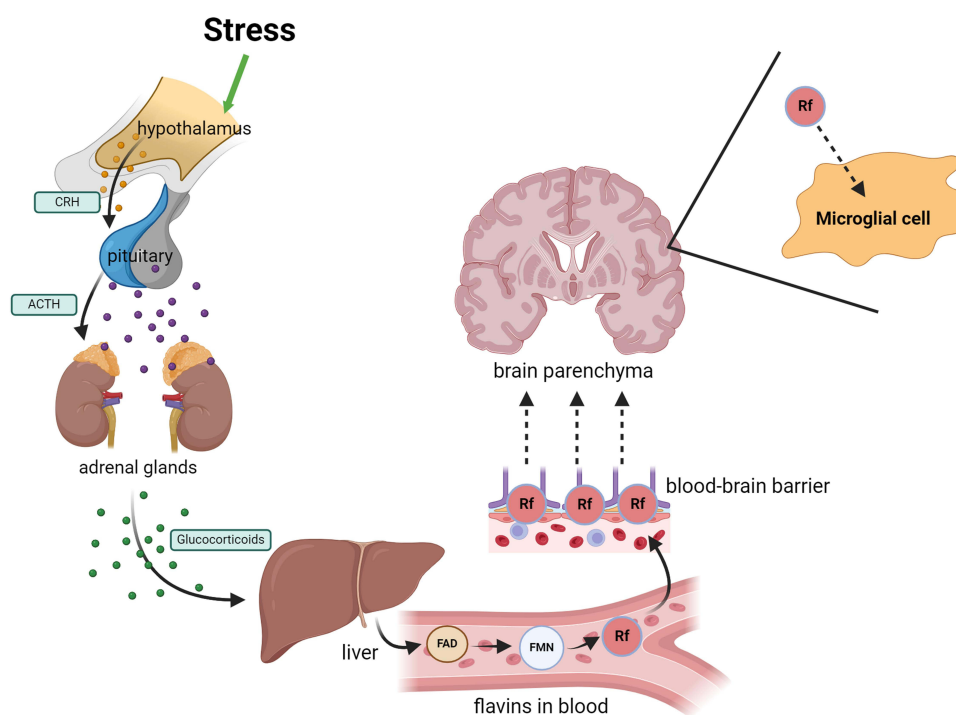


Figure 2 Paradigm for increased nutrient availability to microglia during stress. Psychological stress induces a cascade of events that may culminate with increased riboflavin (Rf) availability to cells of the brain parenchyma. A microglial cell, the main focus of this text, is depicted as an example. The proposed increase in Rf for microglial utilization may be important for the development of depressive phenotypes. Not shown here, is the activation of the hypothalamic-pituitary-adrenal axis by cytokines in the hypothalamus or pituitary. A cytokine-dependent elevation in glucocorticoids would presumably initiate the same release of hepatic Rf. Adrenocorticotropic hormone (ACTH); Corticotropin-releasing hormone (CRH); Flavin adenine dinucleotide (FAD); Flavin mononucleotide (FMN); Riboflavin (Rf). Created in BioRender: Nemeth, D (2024) BioRender.com/t06q366.

(NOX1) were increased in patients with MDD and were positively correlated with Hamilton Rating Scale for Depression (HAM-D) scores.¹²⁴ The finding of increased NOX1 is consistent with the conclusion of a meta-analysis that found increased ROS in MDD.¹²⁵ When taken together, the studies described above suggest that blood flavin levels are specifically increased in stress-susceptible animals. In turn, increased flavins and stress susceptibility appear to be linked with increases in the level or activity of flavoproteins expressed in immune cells that produce ROS.

How might these factors, namely flavins, redox status, and IL-1 β , be related and do they have a causal influence on stress sensitivity and depression? Tight connections between inflammation and cellular redox status are well-known, with studies demonstrating that ROS at the proper concentration typically induce molecular changes that are supportive of pro-inflammatory activities.¹²⁶ Since flavins are cofactors for ROS-generating enzymes, an increase in circulating flavins during states of psychological stress may heighten stress sensitivity through redox-based facilitation of cytokine (especially IL-1 β) generation. In turn, elevated cytokines modulate neural activity in brain regions that are involved in depression. In leukocytes, flavoprotein-dependent ROS (generally H₂O₂) formation causes oxidation of redox-sensitive cysteine residues in proteins or protein complexes that, when oxidized, may promote IL-1 β transcription, maturation, or release (Figure 3).

Specifically, ROS stimulate NF- κ B-dependent transcription of several cytokines, including IL1b, and chemokines. While several studies show that ROS generation by NADPH oxidases^{127,128} and other flavoproteins, including xanthine oxidase/dehydrogenase (XDH)^{129,130} and monoamine oxidase b (MAO-B),¹³¹ are permissive towards IL-1 β production, there is mixed evidence for the importance of ROS in aiding the conversion of pro-IL-1 β to mature IL-1 β via NLRP3 inflammasomes.¹³² Should ROS production by flavoproteins not prove to be responsible for promoting inflammasome oligomerization or function, other redox-related mechanisms of flavoproteins may be relevant, including oxidative phosphorylation-dependent creatine synthesis.¹³³ Mature IL-1 β release is enhanced by ROS.¹³⁴ It is known that cysteine residues in gasdermin D are sensitive to modifications.^{135–137} ROS are important for palmitoylation of gasdermin D cysteine residue 191 and this is essential for channel formation.¹³⁶

The importance of Rf availability as an IL-1 β -regulating factor was shown in a study by Mazur-Bialy et al (2015),¹³⁸ who found that lipopolysaccharide- (LPS) stimulated RAW 264.7 macrophage cells cultured in media with an Rf concentration of 3.1 nM, (representative of moderate Rf deficiency), secreted less IL-1 β than cells cultured in media containing 10.4 nM Rf. Additional evidence comes from studies demonstrating that flavins, which are more available to leukocytes during stress due to increased mobilization from hepatic tissue, serve as cofactors for ROS-producing enzymes needed for IL-1 β transcription, maturation, release, and IL-1 β /IL-1R1 signaling; moreover, canonical signaling downstream of IL-1R1 requires NADPH oxidase activity and ROS formation.^{127,128}

Increased peripheral cytokines transduce signals to the CNS. The above-described mechanisms likely occur in a parallel manner within the brain parenchyma, thereby contributing to immune-mediated neuronal stress sensitization on two fronts. During stress, in addition to being taken up by peripheral leukocytes, the excess Rf released into blood likely crosses the BBB endothelium (Figure 2). Transcriptomic analysis of brain tissue from C57BL/6 mice subjected to a social-defeat stress paradigm,¹⁰⁸ detected elevated hippocampal mRNA expression of the Rf transporter SLC52A3. Single-cell sequencing data¹³⁹ indicates that hippocampal SLC52A3 is almost exclusively expressed by endothelial cells. Therefore, it is possible that stress enhances Rf transport into the brain parenchyma. Extracellular Rf in the brain parenchyma is available for import into various cell types, including the innate immune cells of the CNS, microglia.

Increased levels of cytokines in the periphery lead to the transmission of inflammatory signals to the CNS via afferent pathways, causing microglial reactivity. Reactive microglia produce more IL-1 β , and microglial to neuronal IL-1 β /IL-1R1 signaling can have a variety of depression-related effects that are mediated by specific populations of neurons, as described in the Introduction. Although IL-1 β maturation and release by microglia may be less dependent on caspase-1 activity than IL-1 β production by peripheral leukocytes,¹⁴⁰ increased incorporation of Rf into microglial flavoproteins may enhance IL-1 β maturation in microglia as well. Evidence suggests that iNOS,¹⁴¹ NADPH oxidase,^{142,143} KMO,¹⁴⁴ and XDH^{145,146} are important for microglial-dependent inflammation. Microglial reactivity can also be stimulated by elevated astrocytic MAO-B.¹⁴⁷ Furthermore, RFK is upregulated during and supports CNS inflammation. Zhang et al (2023)¹⁴⁸ found that RFK is detectable through immunohistochemistry in microglia, but not in neurons or astrocytes. In their study, LPS exposure increased RFK protein in BV2 microglial cells, as well as in mouse cortex and hippocampus.

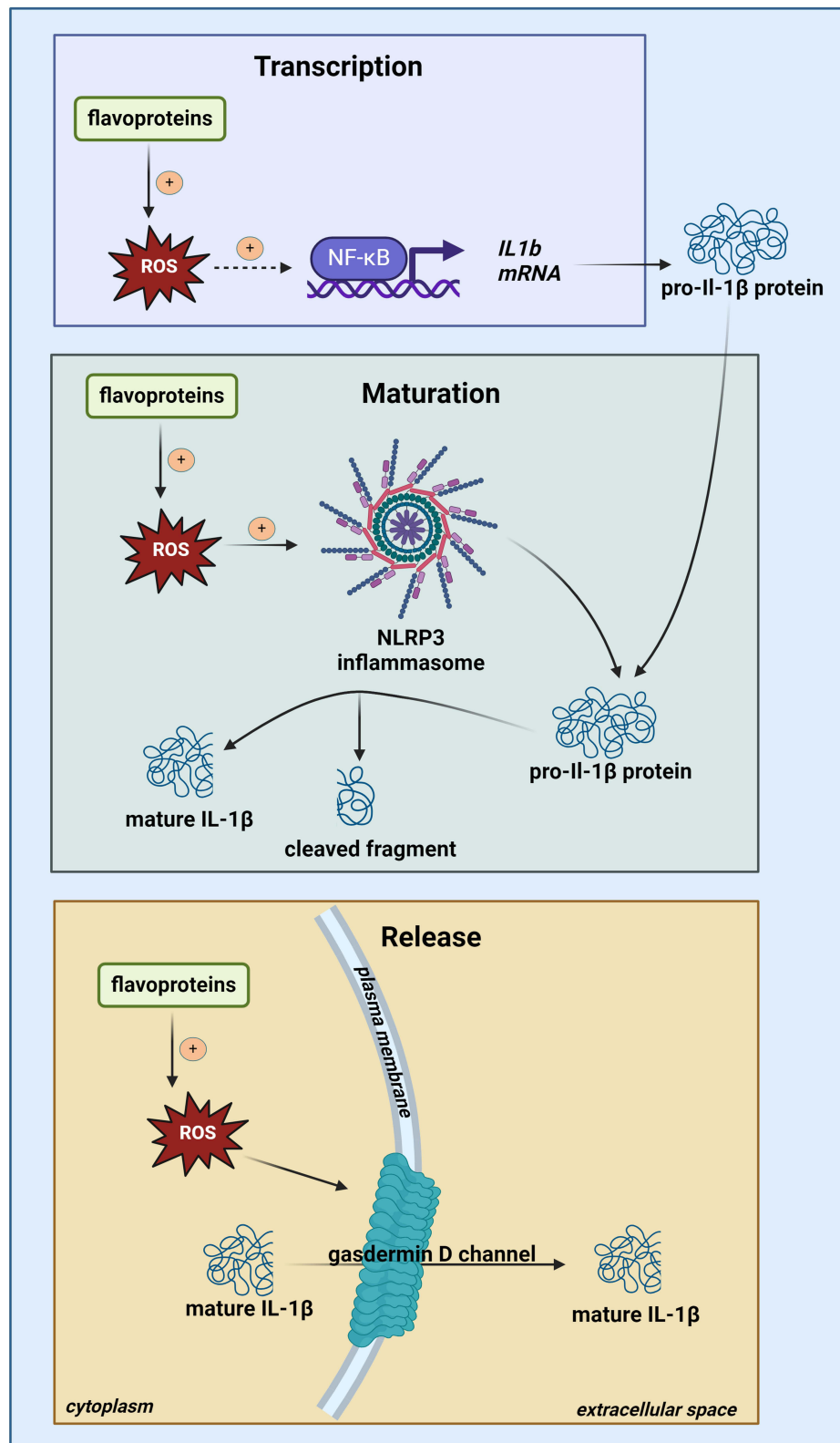


Figure 3 Flavoprotein-dependent reactive oxygen species production is essential for proper IL-1 β expression by immune cells. The majority of enzymes that produce the reactive oxygen species (ROS) superoxide and hydrogen peroxide are flavoproteins. Significantly higher than ambient ROS levels enhances transcription by nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) through redox modification of upstream signaling regulators. Of these transcribed genes, IL1b mRNA is translated to pro-IL-1 β protein, which is cleaved to a less massive IL-1 β protein. This is typically achieved by caspase 1 enzymes associated with NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasomes. The role of ROS in pro-IL-1 β processing to its mature form by NLRP3 inflammasomes is unclear.¹³² However, evidence has come to light in that ROS are necessary for the release of IL-1 β .¹³⁵ Release of mature IL-1 β to the extracellular space is required for its signaling. Created in BioRender. Nemeth, D (2024) BioRender.com/j94p705.

Furthermore, siRNA-mediated knockdown of RFK in both BV2 cells and in primary microglia showed that RFK was important for several pro-inflammatory effects of LPS. The ability of RFK siRNA to attenuate the effects of LPS was especially pronounced in primary microglia, where a decrease in IL-1 β mRNA was detected upon RFK knockdown. Knockdown also blocked an increase in secreted IL-1 β protein in BV2 culture supernatant, thus highlighting that microglial IL-1 β production is sensitive to RFK status.

Translational Implications

Biomarker Prospects of Flavins

While flavins may play a role in pathological neuroimmune processes through their function as cofactors in ROS and nitric oxide production, flavins lack specificity to serve as diagnostic biomarkers for MDD. This is because, as noted in earlier in this manuscript, a collection of insults (including infection, inflammation, psychological stress, and other biological stressors) can cause a transient increase in circulating flavins. Another issue is that flavin levels can actually be decreased in MDD patients.^{43,149} Infection^{44,45,117} and elevated tumor necrosis factor-alpha¹⁵⁰ can, respectively, stimulate urinary flavin excretion and decrease intestinal Rf uptake. Chronic glucocorticoid¹¹⁹ exposure also decreases circulating levels. Therefore, over an extended period of time under stressful conditions, it would be expected that circulating flavin levels will decrease relative to levels found in healthy states. Decreased circulating or solid tissue levels of one or more flavin, or biochemical tests of erythrocyte FAD status (an increased erythrocyte glutathione reductase activation coefficient), have not only been reported in MDD patients, but have also been observed in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome,¹⁵¹ Long COVID,¹⁷¹ HIV,¹⁵² Crohn's Disease or Ulcerative Colitis,^{153,154} and chronic obstructive pulmonary disease.¹⁵⁵ However, plasma FAD was decreased shortly (around 2 days) after elective knee surgery and rebounded to pre-surgical levels by one week post-surgery,¹⁵⁶ representing an exception to decreases after prolonged stressors. These findings as a whole imply that changes in flavin abundance may be a common finding across multiple disease states and cannot generally help to distinguish between illnesses or health conditions. As discussed by Berk (2023)¹⁵⁷ there are numerous types of biomarkers that are clinically-relevant, and it is possible for flavins to serve as biomarkers for purposes unrelated to MDD diagnosis. For example, specific levels or changes in flavins in responders to therapies can be explored for predictive potential. In a small recent study, FMN levels at baseline were correlated with improvements in depression symptoms after intermittent theta burst stimulation on the dorsolateral prefrontal cortex.¹⁷²

Genetic variants in flavin acquisition and/or processing genes could also potentially serve as biomarkers for therapeutic efficacy. In addition to contributing to stress sensitization (Figure 4), it is possible that flavins, RFK, and ROS-producing flavoproteins also influence antidepressant efficacy. The involvement of these factors in microglial redox-dependent IL-1 β signaling to neurons offers a plausible explanation for the findings of Ji et al (2013).¹⁵⁸ Ji et al performed a genome-wide association study to assess genetic factors associated with clinical responsiveness to the SSRIs citalopram and escitalopram in MDD. No genetic variants were significant following false-discovery rate correction. However, a single nucleotide polymorphism (SNP) (rs11144870) in intron 2 of the RFK gene was the most significantly associated SNP detected ($P = 1.04 \times 10^{-6}$) and was linked with a poorer 8 week therapeutic response. The authors probed the molecular impact of this SNP by introducing it into cultured human cell lines, where it was found to increase RFK promoter activity in a luciferase assay relative to cells without the SNP. This suggests that higher RFK transcription decreases the effectiveness of citalopram and escitalopram in MDD by increasing cellular Rf utilization. The presence of the SNP could conceivably increase microglial IL-1 β production to a greater extent in SNP carriers. Higher brain IL-1 β levels in SNP carriers may cause a less effective SSRI response through stronger induction of p38 α MAPK phosphorylation in IL-1R1-expressing serotonin neurons. An alternative hypothesis is that the presence of the SNP strengthens neural circuits involved in rumination or other depressive symptoms, by increasing neuronal Rf usage. Overall, while the above findings are interesting, further research is needed to increase confidence for the analysis of FMN levels or RFK SNP rs11144870 to be deemed to have predictive value.

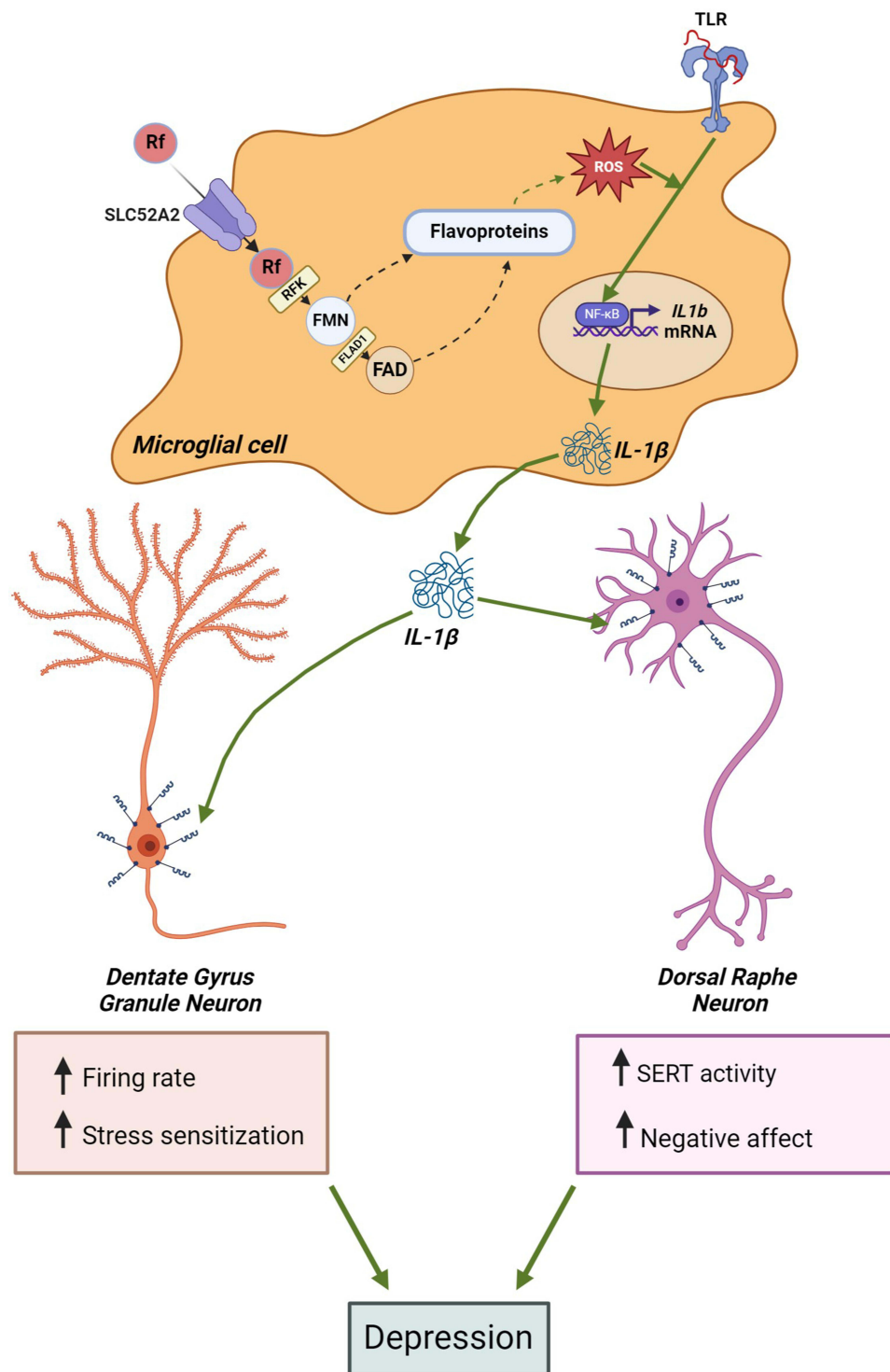


Figure 4 Riboflavin is an important factor for stress sensitization and depression via enhancement of microglial IL-1 β production and neuronal IL-1RI signaling. As discussed earlier, microglia may develop glucocorticoid resistance during periods of increased psychological stress and may contribute to stress sensitization by increasing IL-1 β . Concurrently, glucocorticoids stimulate Rf release from liver storage sites and this activity may lead to increased brain parenchymal riboflavin (Rf). Microglial Rf utilization supports flavoprotein-dependent ROS formation, facilitating the transcription and potentially the processing, as well as the release of IL-1 β . IL-1RI is mainly expressed by glutamatergic neurons of the dentate gyrus, as well as serotonergic neurons of the dorsal raphe nucleus. Stimulation of IL-1RI on these neurons leads to differential effects that contribute to depression. Dentate gyrus glutamatergic neuronal IL-1RI stimulation causes stress sensitization by supporting the encoding of representations of stressful experiences, whereas activation of IL-1RI on serotonergic dorsal raphe neurons decreases extracellular serotonin. Diminished extracellular serotonin can induce a negative affect. Toll-like receptor (TLR). Created in BioRender. Nemeth, D (2024) BioRender.com/i83f212.

Pharmacological Strategies

Drugs that lower Rf availability to microglia and leukocytes have the potential to mitigate stress-induced IL-1 β signaling. Intriguingly, tricyclic antidepressants (TCAs) (eg, imipramine) have been found to decrease flavin content and Rf conversion to active flavins in the brain and in other tissues.^{46,55} This action has been proposed to be due to the structural similarity between Rf and TCAs. Hamilton et al (2020)⁴⁶ found that imipramine treatment decreased serum FAD and ventral hippocampus Rf in stress-susceptible mice and alleviated behavioral deficits. Lowering flavin availability to cells of the immune system could be an unappreciated anti-inflammatory mechanism of TCAs that operates in conjunction with the antidepressant effect of increasing extracellular serotonin through SERT antagonism.

Careful attention is needed for considering pharmacological strategies to help MDD patients, as treatment of MDD patients with broad irreversible inhibitors of flavoproteins, such as diphenylethylidenehydrazinium, could result in unintended systemic effects. Targeting specific flavoproteins involved in ROS generation is a more preferable strategy. Inhibitors of specific flavoproteins that can reduce ROS and inflammation are already used clinically for MDD (eg, the MAO inhibitor selegiline) and other disorders, including gout (eg, the XDH inhibitor febuxostat). MAO inhibitors can diminish ROS production and IL-1 β release by peripheral leukocytes, but the main expressers of MAOs in the CNS are astrocytes³⁵ and neurons.¹⁵⁹ MAO inhibitors would thus not be expected to alter microglial IL-1 β release due to suppression of microglial ROS production, but could dampen microglial inflammatory processes supported by elevated astrocytic MAO-B. Since XDH is expressed by microglia,¹⁴⁵ an XDH inhibitor could be a strategy to explore for depression. Allopurinol and febuxostat decreased immobility time of Swiss Albino mice in the forced swim test,¹⁶⁰ although the mice in that study did not undergo a stress paradigm.

Another approach that could be exploited for inflammation-related depression is the design of drugs that antagonize the physical interaction between RFK and specific client flavoproteins expressed by microglia. This class of drug was proposed by Shan et al (2024)¹⁶¹ as an anti-inflammatory strategy. RFK is known to engage in physical interactions with other proteins to stimulate ROS and nitric oxide formation.^{161–165} Therefore, such drugs could also prove useful for impairing ROS-dependent IL-1 β release. These RFK-interaction antagonists would also need to be tested to ensure that they do not have the off-target effect of inhibiting flavoproteins involved in supporting cellular antioxidants (eg, glutathione reductase).

Lastly, an unexpected treatment possibility for inflammation-associated depression may come in the form of supplementation with high doses of flavins. Flavin supplementation has been carried out in both pre-clinical and clinical trials, where it has mostly demonstrated anti-inflammatory effects ([Supplementary Table 1](#)). Intriguingly, dietary intake of Rf has a negative association with depression risk.^{166,167} These findings seem to contradict the hypothesis that increased flavin availability is supportive of inflammation-linked depression. One possibility is that dietary flavin availability shows a non-linear relationship with depression, which is supported by the findings of Wu et al (2023).¹⁶⁶ In this scenario, a severe lack of Rf can lead to molecular changes that are linked with depression, such as oxidative stress¹⁶⁸ and altered endocrine responsiveness; Optimal Rf intake would promote homeostatic functions; Mild to moderate increases in flavin availability may elevate inflammatory potential to permit pathological changes associated with depression under conditions of psychological stress; Finally, significantly elevated flavin availability, achieved via clinically-supervised supplementation, may attenuate inflammation. Pharmacologically elevated flavins can decrease RFK mRNA expression,¹⁴⁸ abrogate inflammation-induced increases in IL-1 β mRNA and protein,¹⁴⁸ and inhibit NLRP3 inflammasome-mediated processes by interfering with caspase 1 activity.¹⁶⁹

Conclusion

The intricate interplay between flavins, flavoproteins, and IL-1 β /IL-1R1 signaling within the neuroimmune landscape underscores their pivotal roles in stress sensitization and MDD. Increased HPA axis activity downstream of psychological stress alters flavin availability, influencing redox-based mechanisms within leukocytes and microglial cells that, respectively, contribute to elevated peripheral and CNS cytokine production. IL-1 β production is specifically influenced by these changes, due to the potential redox sensitivity of the NLRP3 inflammasome and gasdermin D. IL-1 β /IL-1R1 signaling is a central factor in promoting stress sensitization, depression-like sickness behavior, and decreased serotonin availability. Via their

support of several ROS-producing flavoproteins, flavins serve as a metabolic bridge linking psychological stress, redox alterations, immune system reactivity, IL-1 β /IL-1R1-dependent neuronal modulation, and depression. Contrary to what may occur during the development of depressive conditions, levels of certain flavins may be decreased in MDD. This can be explained by the influence of cytokines and glucocorticoids on flavin absorption and urinary excretion.

A number of health conditions are mentioned in the Biomarker Prospects of Flavins subsection that are linked with decreased flavins. A key limitation to our proposed mechanism on the contribution of flavins to stress sensitization and MDD development is that it does not account for how flavin levels and cellular utilization may vary based on lifestyle factors and comorbid conditions. While we have touched upon the relevance of nutrition, we cannot state with certainty how lifestyle factors, such as alcohol or drug abuse, or comorbid conditions, (eg, autoimmune disease), will influence the applicability of the concepts discussed in this review. Based upon there being decreased flavin levels in several conditions, as well as the literature we outlined in the preceding sections, the directionality of changes in flavins appears to be temporally influenced, with short-term stressors increasing flavins and long-term stressors decreasing flavins. These aspects make flavins a poor diagnostic marker of any particular condition. There is a possibility that flavin levels could become biomarkers predicting or answering other questions important for MDD treatment.

The suggested association between genetic variation in the RFK gene and citalopram responsiveness highlights the potential for identifying treatment responders based on personalized screening of genes involved in flavin metabolism to enhance therapeutic outcomes. However, justification for clinical implementation of such a screen requires further validation in a larger study cohort in order to obtain a level of significance greater than the false-discovery rate threshold. Another finding that has been replicated in multiple studies is the antagonistic effect of TCAs on tissue flavins. It is currently unclear if any of the antidepressant effects of imipramine and other TCAs are related to flavin-lowering effects. Experiments on SERT-mutants insensitive to the antidepressant effects of TCAs, similar to the concept of SERT Met172 mice,¹⁷³ could help to address this question.

We recommend investigating drugs that inhibit ROS- and nitric oxide-producing flavoproteins or their interaction with flavin processing proteins,¹⁶³ as well as studies examining flavin supplementation for depression. Understanding the nuanced involvement of flavins and flavoproteins in neuroimmune processes not only illuminates the pathophysiology of MDD but also offers novel avenues for promising therapeutics aimed at alleviating stress-induced depressive states. We hope that the current review inspires additional research into these areas.

Acknowledgments

Graphical abstract is created in BioRender. Nemeth, D. (2024) BioRender.com/a49o500.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by the National Institute of Neurological Disorders and Stroke (NINDS) NS116914. Our sponsor was not involved in the development or submission of this paper.

Disclosure

The authors declare no competing interests in this work.

References

1. Ehlman DC, Yard E, Stone DM, Jones CM, Mack KA. Changes in Suicide Rates — United States, 2019 and 2020. *MMWR Morb Mortal Wkly Rep.* 2022;71(8):306–312. doi:10.15585/mmwr.mm7108a5
2. Kim H, Jeong W, Kwon J, Kim Y, Park EC, Jang SI. Association between depression and the risk of Alzheimer's disease using the Korean National Health Insurance Service-Elderly Cohort. *Sci Rep.* 2021;11(1):22591. doi:10.1038/s41598-021-02201-6

3. Sáiz-Vázquez O, Gracia-García P, Ubillos-Landa S, et al. Depression as a Risk Factor for Alzheimer's Disease: a Systematic Review of Longitudinal Meta-Analyses. *JCM*. 2021;10(9):1809. doi:10.3390/jcm10091809
4. Berk M, Köhler-Forsberg O, Turner M, et al. Comorbidity between major depressive disorder and physical diseases: a comprehensive review of epidemiology, mechanisms and management. *World Psychiatry*. 2023;22(3):366–387. doi:10.1002/wps.21110
5. Cipriani A, Furukawa TA, Salanti G, et al. Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: a systematic review and network meta-analysis. *Lancet*. 2018;391(10128):1357–1366. doi:10.1016/S0140-6736(17)32802-7
6. Lagerberg T, Fazel S, Sjölander A, Hellner C, Lichtenstein P, Chang Z. Selective serotonin reuptake inhibitors and suicidal behaviour: a population-based cohort study. *Neuropsychopharmacol*. 2022;47(4):817–823. doi:10.1038/s41386-021-01179-z
7. McIntyre RS, Alsuwaidan M, Baune BT, et al. Treatment-resistant depression: definition, prevalence, detection, management, and investigational interventions. *World Psychiatry*. 2023;22(3):394–412. doi:10.1002/wps.21120
8. Capuron L, Ravaut A, Dantzer R. Early Depressive Symptoms in Cancer Patients Receiving Interleukin 2 and/or Interferon Alfa-2b Therapy. *JCO*. 2000;18(10):2143–2151. doi:10.1200/JCO.2000.18.10.2143
9. Musselman DL, Lawson DH, Gumnick JF, et al. Paroxetine for the Prevention of Depression Induced by High-Dose Interferon Alfa. *N Engl J Med*. 2001;344(13):961–966. doi:10.1056/NEJM200103293441303
10. Brydon L, Edwards S, Jia H, et al. Psychological stress activates interleukin-1 β gene expression in human mononuclear cells. *Brain Behav Immun*. 2005;19(6):540–546. doi:10.1016/j.bbi.2004.12.003
11. Coussons-Read ME, Okun ML, Nettles CD. Psychosocial stress increases inflammatory markers and alters cytokine production across pregnancy. *Brain Behav Immun*. 2007;21(3):343–350. doi:10.1016/j.bbi.2006.08.006
12. Yang C, Tiemessen KM, Bosker FJ, Wardenaar KJ, Lie J, Schoevers RA. Interleukin, tumor necrosis factor- α and C-reactive protein profiles in melancholic and non-melancholic depression: a systematic review. *J Psychosomatic Res*. 2018;111:58–68. doi:10.1016/j.jpsychores.2018.05.008
13. Goshen I, Kreisel T, Ben-Menachem-Zidon O, et al. Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression. *Mol Psychiatry*. 2008;13(7):717–728. doi:10.1038/sj.mp.4002055
14. Koo JW, Duman RS. IL-1 β is an essential mediator of the antineurogenic and anhedonic effects of stress. *Proc Natl Acad Sci USA*. 2008;105(2):751–756. doi:10.1073/pnas.0708092105
15. Zhu CB, Lindler KM, Owens AW, Daws LC, Blakely RD, Hewlett WA. Interleukin-1 Receptor Activation by Systemic Lipopolysaccharide Induces Behavioral Despair Linked to MAPK Regulation of CNS Serotonin Transporters. *Neuropsychopharmacol*. 2010;35(13):2510–2520. doi:10.1038/npp.2010.116
16. Sims JE, Gayle MA, Slack JL, et al. Interleukin 1 signaling occurs exclusively via the type I receptor. *Proc Natl Acad Sci USA*. 1993;90(13):6155–6159. doi:10.1073/pnas.90.13.6155
17. Song A, Zhu L, Gorantla G, et al. Salient type 1 interleukin 1 receptor expression in peripheral non-immune cells. *Sci Rep*. 2018;8(1):723. doi:10.1038/s41598-018-19248-7
18. Liu X, Nemeth DP, McKim DB, et al. Cell-Type-Specific Interleukin 1 Receptor 1 Signaling in the Brain Regulates Distinct Neuroimmune Activities. *Immunity*. 2019;50(2):317–333.e6. doi:10.1016/j.immuni.2018.12.012
19. Yabuuchi K, Minami M, Katsumata S, Satoh M. Localization of type I interleukin-1 receptor mRNA in the rat brain. *Mol Brain Res*. 1994;27(1):27–36. doi:10.1016/0169-328X(94)90180-5
20. Nemeth DP, Quan N. Modulation of Neural Networks by Interleukin-1. *BPL*. 2021;7(1):17–32. doi:10.3233/BPL-200109
21. Mohankumar PS, Thyagarajan S, Quadri SK. Interleukin-1 β increases 5-hydroxyindoleacetic acid release in the hypothalamus in vivo. *Brain Res Bull*. 1993;31(6):745–748. doi:10.1016/0361-9230(93)90151-Z
22. Shintani F, Kanba S, Nakaki T, et al. Interleukin-1 beta augments release of norepinephrine, dopamine, and serotonin in the rat anterior hypothalamus. *J Neurosci*. 1993;13(8):3574–3581. doi:10.1523/JNEUROSCI.13-08-03574.1993
23. Takahashi A, Aleyasin H, Stavarache MA, et al. Neuromodulatory effect of interleukin 1 β in the dorsal raphe nucleus on individual differences in aggression. *Mol Psychiatry*. 2022;27(5):2563–2579. doi:10.1038/s41380-021-01110-4
24. Zhu CB, Blakely RD, Hewlett WA. The Proinflammatory Cytokines Interleukin-1beta and Tumor Necrosis Factor-Alpha Activate Serotonin Transporters. *Neuropsychopharmacol*. 2006;31(10):2121–2131. doi:10.1038/sj.npp.1301029
25. Lienhart WD, Gudipati V, Macheroux P. The human flavoproteome. *Arch Biochem Biophys*. 2013;535(2):150–162. doi:10.1016/j.abb.2013.02.015
26. McNulty H, Pentieva K, Ward M. Causes and Clinical Sequelae of Riboflavin Deficiency. *Annu Rev Nutr*. 2023;43(1):101–122. doi:10.1146/annurev-nutr-061121-084407
27. Brady PS, Hoppel CL. Hepatic peroxisomal and mitochondrial fatty acid oxidation in the riboflavin-deficient rat. *Biochem J*. 1985;229(3):717–721. doi:10.1042/bj2290717
28. Curtabbi A, Guarás A, Cabrera-Alarcón JL, et al. Regulation of respiratory complex I assembly by FMN cofactor targeting. *Redox Biol*. 2024;69:103001. doi:10.1016/j.redox.2023.103001
29. Walker WH, Singer TP. Identification of the Covalently Bound Flavin of Succinate Dehydrogenase as 8 α -(Histidyl) Flavin Adenine Dinucleotide. *J Biol Chem*. 1970;245(16):4224–4225. doi:10.1016/S0021-9258(18)62907-2
30. Mazur-Bialy AI, Buchala B, Plytycz B. Riboflavin deprivation inhibits macrophage viability and activity – a study on the RAW 264.7 cell line. *Br J Nutr*. 2013;110(3):509–514. doi:10.1017/S0007114512005351
31. Rivlin RS, Wolf G. Diminished Responsiveness to Thyroid Hormone in Riboflavin-deficient Rats. *Nature*. 1969;223(5205):516–517. doi:10.1038/223516a0
32. Henderson LM, Koski RE, D'angeli F. THE ROLE OF RIBOFLAVIN AND VITAMIN B6 IN TRYPTOPHAN METABOLISM. *J Biol Chem*. 1955;215(1):369–376. doi:10.1016/S0021-9258(18)66045-4
33. Mason M. THE METABOLISM OF TRYPTOPHAN IN RIBOFLAVIN DEFICIENT RATS. *J Biol Chem*. 1953;201(2):513–518. doi:10.1016/S0021-9258(18)66205-2
34. Sylianco CYL, Berg CP. The Effect of Riboflavin Deficiency upon the Metabolism of Tryptophan by Liver and Kidney Tissue. *J Biol Chem*. 1959;234(4):912–917. doi:10.1016/S0021-9258(18)70201-9

35. Fitzgerald LW, Kaplinsky L, Kimelberg HK. Serotonin Metabolism by Monoamine Oxidase in Rat Primary Astrocyte Cultures. *Journal of Neurochemistry*. 1990;55(6):2008–2014. doi:10.1111/j.1471-4159.1990.tb05789.x
36. Prah A, Purg M, Stare J, Vianello R, Mavri J. How Monoamine Oxidase A Decomposes Serotonin: an Empirical Valence Bond Simulation of the Reactive Step. *J Phys Chem B*. 2020;124(38):8259–8265. doi:10.1021/acs.jpcc.0c06502
37. Sterner RT, Price WR. Restricted riboflavin: within-subject behavioral effects in humans. *The American Journal of Clinical Nutrition*. 1973;26(2):150–160. doi:10.1093/ajcn/26.2.150
38. Leone P, Tolomeo M, Piancone E, et al. Mimicking human riboflavin responsive neuromuscular disorders by silencing *flad-1* gene in *C. ELEGANS*: alteration of vitamin transport and cholinergic transmission. *IUBMB Life*. 2022;74(7):672–683. doi:10.1002/iub.2553
39. Liuzzi VC, Giancaspero TA, Gianazza E, Banfi C, Barile M, De Giorgi C. Silencing of FAD synthase gene in *Caenorhabditis elegans* upsets protein homeostasis and impacts on complex behavioral patterns. *Biochim Biophys Acta - General Subjects*. 2012;1820(4):521–531. doi:10.1016/j.bbagen.2012.01.012
40. Manole A, Jaunmuktane Z, Hargreaves I, et al. Clinical, pathological and functional characterization of riboflavin-responsive neuropathy. *Brain*. 2017;140(11):2820–2837. doi:10.1093/brain/awx231
41. Masschelin PM, Saha P, Ochsner SA, et al. Vitamin B2 enables regulation of fasting glucose availability. *eLife*. 2023;12:e84077. doi:10.7554/eLife.84077
42. Peretti PO, Baird M. Experimental studies of food selective behavior in squirrel monkeys fed on riboflavin deficient diet. *J Nutr Sci Vitaminol*. 1975;21(3):199–206. doi:10.3177/jnsv.21.199
43. Mocking RJT, Naviaux JC, Li K, et al. Metabolic features of recurrent major depressive disorder in remission, and the risk of future recurrence. *Transl Psychiatry*. 2021;11(1):37. doi:10.1038/s41398-020-01182-w
44. Bamji MS, Bhaskaram P, Jacob CM. Urinary Riboflavin Excretion and Erythrocyte Glutathione Reductase Activity in Preschool Children Suffering from Upper Respiratory Infections and Measles. *Ann Nutr Metab*. 1987;31(3):191–196. doi:10.1159/000177268
45. Brijlal S, Lakshmi AV. Tissue distribution and turnover of [³H]riboflavin during respiratory infection in mice. *Metabolism*. 1999;48(12):1608–1611. doi:10.1016/S0026-0495(99)90253-6
46. Hamilton PJ, Chen EY, Tolstikov V, et al. Chronic stress and antidepressant treatment alter purine metabolism and beta oxidation within mouse brain and serum. *Sci Rep*. 2020;10(1):18134. doi:10.1038/s41598-020-75114-5
47. Anrather J, Racchumi G, Iadecola C. NF- κ B Regulates Phagocytic NADPH Oxidase by Inducing the Expression of gp91. *J Biol Chem*. 2006;281(9):5657–5667. doi:10.1074/jbc.M506172200
48. Xie QW, Kashiwabara Y, Nathan C. Role of transcription factor NF-kappa B/Rel in induction of nitric oxide synthase. *J Biol Chem*. 1994;269(7):4705–4708. doi:10.1016/S0021-9258(17)37600-7
49. Abcouwer SF, Shanmugam S, Gomez PF, et al. Effect of IL-1 β on Survival and Energy Metabolism of R28 and RGC-5 Retinal Neurons. *Invest Ophthalmol Vis Sci*. 2008;49(12):5581. doi:10.1167/iovs.07-1032
50. Tatsumi T, Matoba S, Kawahara A, et al. Cytokine-induced nitric oxide production inhibits mitochondrial energy production and impairs contractile function in rat cardiac myocytes. *J Am College Cardiol*. 2000;35(5):1338–1346. doi:10.1016/S0735-1097(00)00526-X
51. Zell R, Geck P, Werdan K, Boekstegers P. TNF-alpha and IL-1 alpha inhibit both pyruvate dehydrogenase activity and mitochondrial function in cardiomyocytes: evidence for primary impairment of mitochondrial function. *Mol Cell Biochem*. 1997;177(1/2):61–67. doi:10.1023/A:1006896832582
52. Eitner A, Müller S, König C, et al. Inhibition of Inducible Nitric Oxide Synthase Prevents IL-1 β -Induced Mitochondrial Dysfunction in Human Chondrocytes. *IJMS*. 2021;22(5):2477. doi:10.3390/ijms22052477
53. Gavillet M, Allaman I, Magistretti PJ. Modulation of astrocytic metabolic phenotype by proinflammatory cytokines. *Glia*. 2008;56(9):975–989. doi:10.1002/glia.20671
54. Peruzzotti-Jametti L, Willis CM, Krzak G, et al. Mitochondrial complex I activity in microglia sustains neuroinflammation. *Nature*. 2024;628(8006):195–203. doi:10.1038/s41586-024-07167-9
55. Pinto J, Huang YP, Rivlin RS. Inhibition of Riboflavin Metabolism in Rat Tissues by Chlorpromazine, Imipramine, and Amitriptyline. *J Clin Invest*. 1981;67(5):1500–1506. doi:10.1172/JCI110180
56. Pinto J, Huang YP, Pelliccione N, Rivlin RS. Cardiac sensitivity to the inhibitory effects of chlorpromazine, imipramine and amitriptyline upon formation of flavins. *Biochem Pharmacol*. 1982;31(21):3495–3499. doi:10.1016/0006-2952(82)90632-3
57. Powers HJ. Riboflavin (vitamin B-2) and health. *The American Journal of Clinical Nutrition*. 2003;77(6):1352–1360. doi:10.1093/ajcn/77.6.1352
58. Mosegaard S, Dipace G, Bross P, Carlsen J, Gregersen N, Olsen RKJ. Riboflavin Deficiency—Implications for General Human Health and Inborn Errors of Metabolism. *IJMS*. 2020;21(11):3847. doi:10.3390/ijms21113847
59. Yonezawa A, Masuda S, Katsura T, Inui K. Identification and functional characterization of a novel human and rat riboflavin transporter, RFT1. *Am J Physiol Cell Physiol*. 2008;295(3):C632–C641. doi:10.1152/ajpcell.00019.2008
60. Fujimura M, Yamamoto S, Murata T, et al. Functional Characteristics of the Human Ortholog of Riboflavin Transporter 2 and Riboflavin-Responsive Expression of Its Rat Ortholog in the Small Intestine Indicate Its Involvement in Riboflavin Absorption. *J Nutr*. 2010;140(10):1722–1727. doi:10.3945/jn.110.128330
61. Yamamoto S, Inoue K, Ohta KY, et al. Identification and Functional Characterization of Rat Riboflavin Transporter 2. *J Biochemistry*. 2009;145(4):437–443. doi:10.1093/jb/mvn181
62. Subramanian VS, Ghosal A, Kapadia R, Nabokina SM, Said HM. Molecular Mechanisms Mediating the Adaptive Regulation of Intestinal Riboflavin Uptake Process. *PLoS One*. 2015;10(6):e0131698. doi:10.1371/journal.pone.0131698
63. Yao Y, Yonezawa A, Yoshimatsu H, Masuda S, Katsura T, Inui K. Identification and Comparative Functional Characterization of a New Human Riboflavin Transporter hRFT3 Expressed in the Brain. *J Nutr*. 2010;140(7):1220–1226. doi:10.3945/jn.110.122911
64. Shichinohe N, Kobayashi D, Izumi A, et al. Sequential hydrolysis of FAD by ecto-5' nucleotidase CD73 and alkaline phosphatase is required for uptake of vitamin B2 into cells. *J Biol Chem*. 2022;298(12):102640. doi:10.1016/j.jbc.2022.102640
65. McCormick DB. The Intracellular Localization, Partial Purification, and Properties of Flavokinase from Rat Liver. *J Biol Chem*. 1962;237(3):959–962. doi:10.1016/S0021-9258(18)60400-4

66. Giancaspero TA, Busco G, Panebianco C, et al. FAD Synthesis and Degradation in the Nucleus Create a Local Flavin Cofactor Pool. *J Biol Chem.* 2013;288(40):29069–29080. doi:10.1074/jbc.M113.500066
67. Agrimi G, Russo A, Scarcia P, Palmieri F. The human gene *SLC25A17* encodes a peroxisomal transporter of coenzyme A, FAD and NAD⁺. *Biochem J.* 2012;443(1):241–247. doi:10.1042/BJ20111420
68. Spaan AN, IJlst L, Van Roermund CWT, Wijburg FA, Wanders RJA, Waterham HR. Identification of the human mitochondrial FAD transporter and its potential role in multiple acyl-CoA dehydrogenase deficiency. *Mol Gene Metabol.* 2005;86(4):441–447. doi:10.1016/j.ymgme.2005.07.014
69. Kuang W, Zhang J, Lan Z, et al. *SLC22A14* is a mitochondrial riboflavin transporter required for sperm oxidative phosphorylation and male fertility. *Cell Rep.* 2021;35(3):109025. doi:10.1016/j.celrep.2021.109025
70. Strauss E, Begley TP. Mechanistic Studies on Phosphopantothencycysteine Decarboxylase. *J Am Chem Soc.* 2001;123(26):6449–6450. doi:10.1021/ja016020y
71. Musayev FN, Di Salvo ML, Ko T, Schirch V, Safo MK. Structure and properties of recombinant human pyridoxine 5'-phosphate oxidase. *Protein Sci.* 2003;12(7):1455–1463. doi:10.1110/ps.0356203
72. Gherasim CG, Zaman U, Raza A, Banerjee R. Impeded Electron Transfer From a Pathogenic FMN Domain Mutant of Methionine Synthase Reductase and Its Responsiveness to Flavin Supplementation. *Biochemistry.* 2008;47(47):12515–12522. doi:10.1021/bi8008328
73. Leclerc D, Wilson A, Dumas R, et al. Cloning and mapping of a cDNA for methionine synthase reductase, a flavoprotein defective in patients with homocystinuria. *Proc Natl Acad Sci.* 1998;95(6):3059–3064. doi:10.1073/pnas.95.6.3059
74. Olteanu H, Munson T, Banerjee R. Differences in the Efficiency of Reductive Activation of Methionine Synthase and Exogenous Electron Acceptors between the Common Polymorphic Variants of Human Methionine Synthase Reductase. *Biochemistry.* 2002;41(45):13378–13385. doi:10.1021/bi020536s
75. Olteanu H, Banerjee R. Human Methionine Synthase Reductase, a Soluble P-450 Reductase-like Dual Flavoprotein, Is Sufficient for NADPH-dependent Methionine Synthase Activation. *J Biol Chem.* 2001;276(38):35558–35563. doi:10.1074/jbc.M103707200
76. Yamada K, Gravel RA, Toraya T, Matthews RG. Human methionine synthase reductase is a molecular chaperone for human methionine synthase. *Proc Natl Acad Sci.* 2006;103(25):9476–9481. doi:10.1073/pnas.0603694103
77. Hirano A, Braas D, Fu YH, Ptáček LJ. FAD Regulates CRYPTOCHROME Protein Stability and Circadian Clock in Mice. *Cell Rep.* 2017;19(2):255–266. doi:10.1016/j.celrep.2017.03.041
78. Tu BP, Ho-Schleyer SC, Travers KJ, Weissman JS. Biochemical Basis of Oxidative Protein Folding in the Endoplasmic Reticulum. *Science.* 2000;290(5496):1571–1574. doi:10.1126/science.290.5496.1571
79. Beutler E. Glutathione Reductase: stimulation in Normal Subjects by Riboflavin Supplementation. *Science.* 1969;165(3893):613–615. doi:10.1126/science.165.3893.613
80. Jackson MR, Melideo SL, Jorns MS. Human Sulfide:Quinone Oxidoreductase Catalyzes the First Step in Hydrogen Sulfide Metabolism and Produces a Sulfane Sulfur Metabolite. *Biochemistry.* 2012;51(34):6804–6815. doi:10.1021/bi300778t
81. Landry AP, Moon S, Bonanata J, Cho US, Coitino EL, Banerjee R. Dismantling and Rebuilding the Trisulfide Cofactor Demonstrates Its Essential Role in Human Sulfide Quinone Oxidoreductase. *J Am Chem Soc.* 2020;142(33):14295–14306. doi:10.1021/jacs.0c06066
82. Menendez C, Hacker P, Sonnenfeld M, McConnell R, Rivlin R. Thyroid hormone control of glutathione reductase activity in rat erythrocytes and liver. *Am J Physiol Legacy Content.* 1974;226(6):1480–1483. doi:10.1152/ajplegacy.1974.226.6.1480
83. Pinto J, Rivlin RS. Regulation of formation of covalently bound flavins in liver and cerebrum by thyroid hormones. *Arch Biochem Biophys.* 1979;194(2):313–320. doi:10.1016/0003-9861(79)90623-4
84. Rivlin RS, Menendez C, Langdon RG. Biochemical Similarities Between Hypothyroidism and Riboflavin Deficiency. *Endocrinology.* 1968;83(3):461–469. doi:10.1210/endo-83-3-461
85. Pinto JT, Cooper AJL. From Cholesterologenesis to Steroidogenesis: role of Riboflavin and Flavoenzymes in the Biosynthesis of Vitamin D. *Adv Nutr.* 2014;5(2):144–163. doi:10.3945/an.113.005181
86. Go YM, Chandler JD, Jones DP. The cysteine proteome. *Free Radic Biol Med.* 2015;84:227–245. doi:10.1016/j.freeradbiomed.2015.03.022
87. Sies H, Belousov VV, Chandel NS, et al. Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. *Nat Rev Mol Cell Biol.* 2022;23(7):499–515. doi:10.1038/s41580-022-00456-z
88. Banerjee R, ed. *Redox Biochemistry.* Wiley-Interscience; 2008.
89. Kendler KS, Karkowski LM, Prescott CA. Causal Relationship Between Stressful Life Events and the Onset of Major Depression. *AJP.* 1999;156(6):837–841. doi:10.1176/ajp.156.6.837
90. Biltz RG, Sawicki CM, Sheridan JF, Godbout JP. The neuroimmunology of social-stress-induced sensitization. *Nat Immunol.* 2022;23(11):1527–1535. doi:10.1038/s41590-022-01321-z
91. Auger JP, Zimmermann M, Faas M, et al. Metabolic rewiring promotes anti-inflammatory effects of glucocorticoids. *Nature.* 2024;629(8010):184–192. doi:10.1038/s41586-024-07282-7
92. Frank MG, Thompson BM, Watkins LR, Maier SF. Glucocorticoids mediate stress-induced priming of microglial pro-inflammatory responses. *Brain Behav Immun.* 2012;26(2):337–345. doi:10.1016/j.bbi.2011.10.005
93. Feng X, Zhao Y, Yang T, et al. Glucocorticoid-Driven NLRP3 Inflammasome Activation in Hippocampal Microglia Mediates Chronic Stress-Induced Depressive-Like Behaviors. *Front Mol Neurosci.* 2019;12:210. doi:10.3389/fnmol.2019.00210
94. Perrin AJ, Horowitz MA, Roelofs J, Zunsain PA, Pariante CM. Glucocorticoid Resistance: is It a Requisite for Increased Cytokine Production in Depression? A Systematic Review and Meta-Analysis. *Front Psychiatry.* 2019;10:423. doi:10.3389/fpsy.2019.00423
95. Osimo EF, Pillinger T, Rodriguez IM, Khandaker GM, Pariante CM, Howes OD. Inflammatory markers in depression: a meta-analysis of mean differences and variability in 5,166 patients and 5,083 controls. *Brain Behav Immun.* 2020;87:901–909. doi:10.1016/j.bbi.2020.02.010
96. Nguyen KT, Deak T, Will MJ, et al. Timecourse and corticosterone sensitivity of the brain, pituitary, and serum interleukin-1 β response to acute stress. *Brain Res.* 2000;859(2):193–201. doi:10.1016/S0006-8993(99)02443-9
97. Alcocer-Gómez E, de Miguel M, Casas-Barquero N, et al. NLRP3 inflammasome is activated in mononuclear blood cells from patients with major depressive disorder. *Brain Behav Immun.* 2014;36:111–117. doi:10.1016/j.bbi.2013.10.017
98. Pandey GN, Zhang H, Sharma A, Ren X. Innate immunity receptors in depression and suicide: upregulated NOD-like receptors containing pyrin (NLRPs) and hyperactive inflammasomes in the postmortem brains of people who were depressed and died by suicide. *jpn.* 2021;46(5):E538–E547. doi:10.1503/jpn.210016

99. Taene A, Khalili-Tanha G, Esmaeili A, et al. The Association of Major Depressive Disorder with Activation of NLRP3 Inflammasome, Lipid Peroxidation, and Total Antioxidant Capacity. *J Mol Neurosci.* 2020;70(1):65–70. doi:10.1007/s12031-019-01401-0
100. McKim DB, Weber MD, Niraula A, et al. Microglial recruitment of IL-1 β -producing monocytes to brain endothelium causes stress-induced anxiety. *Mol Psychiatry.* 2018;23(6):1421–1431. doi:10.1038/mp.2017.64
101. Murray CA, Lynch MA. Evidence That Increased Hippocampal Expression of the Cytokine Interleukin-1 β Is a Common Trigger for Age- and Stress-Induced Impairments in Long-Term Potentiation. *J Neurosci.* 1998;18(8):2974–2981. doi:10.1523/JNEUROSCI.18-08-02974.1998
102. Wohleb ES, Patterson JM, Sharma V, Quan N, Godbout JP, Sheridan JF. Knockdown of Interleukin-1 Receptor Type-1 on Endothelial Cells Attenuated Stress-Induced Neuroinflammation and Prevented Anxiety-Like Behavior. *J Neurosci.* 2014;34(7):2583–2591. doi:10.1523/JNEUROSCI.3723-13.2014
103. Yang Y, Xing MJ, Li Y, Zhang HF, Yuan TF, Peng DH. Reduced NLRP3 inflammasome expression in the brain is associated with stress resilience. *Psychoneuroendocrinology.* 2021;128:105211. doi:10.1016/j.psyneuen.2021.105211
104. Alcocer-Gómez E, Ulecia-Morón C, Marín-Aguilar F, et al. Stress-Induced Depressive Behaviors Require a Functional NLRP3 Inflammasome. *Mol Neurobiol.* 2016;53(7):4874–4882. doi:10.1007/s12035-015-9408-7
105. Buttini M, Boddeke H. Peripheral lipopolysaccharide stimulation induces interleukin-1 β messenger RNA in rat brain microglial cells. *Neuroscience.* 1995;65(2):523–530. doi:10.1016/0306-4522(94)00525-A
106. Giulian D, Baker TJ, Shih LC, Lachman LB. Interleukin 1 of the central nervous system is produced by ameboid microglia. *J Exp Med.* 1986;164(2):594–604. doi:10.1084/jem.164.2.594
107. Lehmann ML, Weigel TK, Poffenberger CN, Herkenham M. The Behavioral Sequelae of Social Defeat Require Microglia and Are Driven by Oxidative Stress in Mice. *J Neurosci.* 2019;39(28):5594–5605. doi:10.1523/JNEUROSCI.0184-19.2019
108. DiSabato DJ, Nemeth DP, Liu X, et al. Interleukin-1 receptor on hippocampal neurons drives social withdrawal and cognitive deficits after chronic social stress. *Mol Psychiatry.* 2021;26(9):4770–4782. doi:10.1038/s41380-020-0788-3
109. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci.* 2008;9(1):46–56. doi:10.1038/nrn2297
110. Zhu CB, Carneiro AM, Dostmann WR, Hewlett WA, Blakely RD. p38 MAPK Activation Elevates Serotonin Transport Activity via a Trafficking-independent, Protein Phosphatase 2A-dependent Process. *J Biol Chem.* 2005;280(16):15649–15658. doi:10.1074/jbc.M410858200
111. Baganz NL, Lindler KM, Zhu CB, et al. A requirement of serotonergic p38 α mitogen-activated protein kinase for peripheral immune system activation of CNS serotonin uptake and serotonin-linked behaviors. *Transl Psychiatry.* 2015;5(11):e671–e671. doi:10.1038/tp.2015.168
112. Zunszain PA, Anacker C, Cattaneo A, et al. Interleukin-1 β : a New Regulator of the Kynurenine Pathway Affecting Human Hippocampal Neurogenesis. *Neuropsychopharmacol.* 2012;37(4):939–949. doi:10.1038/npp.2011.277
113. Tong L, Balazs R, Soiampornkul R, Thangnipon W, Cotman CW. Interleukin-1 β impairs brain derived neurotrophic factor-induced signal transduction. *Neurobiol Aging.* 2008;29(9):1380–1393. doi:10.1016/j.neurobiolaging.2007.02.027
114. Tong L, Prieto GA, Kramár EA, et al. Brain-Derived Neurotrophic Factor-Dependent Synaptic Plasticity Is Suppressed by Interleukin-1 β via p38 Mitogen-Activated Protein Kinase. *J Neurosci.* 2012;32(49):17714–17724. doi:10.1523/JNEUROSCI.1253-12.2012
115. Jagot F, Gaston-Breton R, Choi AJ, et al. The parabrachial nucleus elicits a vigorous corticosterone feedback response to the pro-inflammatory cytokine IL-1 β . *Neuron.* 2023;111(15):2367–2382.e6. doi:10.1016/j.neuron.2023.05.009
116. Matsuwaki T, Eskilsson A, Kugelberg U, Jönsson JI, Blomqvist A. Interleukin-1 β induced activation of the hypothalamus–pituitary–adrenal axis is dependent on interleukin-1 receptors on non-hematopoietic cells. *Brain Behav Immun.* 2014;40:166–173. doi:10.1016/j.bbi.2014.03.015
117. Brijljal S, Lakshmi AV, Bamji MS, Suresh P. Flavin metabolism during respiratory infection in mice. *Br J Nutr.* 1996;76(3):453–462. doi:10.1079/BJN19960050
118. Fazekas AG, Sandor T. The influence of corticosteroids on flavin nucleotide biosynthesis in rat liver and kidney. *J Steroid Biochem.* 1976;7(1):29–32. doi:10.1016/0022-4731(76)90160-6
119. Nakamura T, Nomoto N, Yagi R, Oya N. Metabolic Vitamin B Complex Deficiency due to the Administration of Glucocorticoid. *J Vitaminol.* 1970;16(2):89–98. doi:10.5925/jnsv1954.16.89
120. Qiu W, Go KA, Lamers Y, Galea LAM. Postpartum corticosterone and fluoxetine shift the tryptophan-kynurenine pathway in dams. *Psychoneuroendocrinology.* 2021;130:105273. doi:10.1016/j.psyneuen.2021.105273
121. Zolkipli-Cunningham Z, Naviaux JC, Nakayama T, et al. Metabolic and behavioral features of acute hyperpurinergia and the maternal immune activation mouse model of autism spectrum disorder. *PLoS One.* 2021;16(3):e0248771. doi:10.1371/journal.pone.0248771
122. Okuda S, Nishiyama N, Saito H, Katsuki H. Hydrogen peroxide-mediated neuronal cell death induced by an endogenous neurotoxin, 3-hydroxykynurenine. *Proc Natl Acad Sci USA.* 1996;93(22):12553–12558. doi:10.1073/pnas.93.22.12553
123. Wang Q, Zhang M, Ding Y, et al. Activation of NAD(P)H Oxidase by Tryptophan-Derived 3-Hydroxykynurenine Accelerates Endothelial Apoptosis and Dysfunction In Vivo. *Circ Res.* 2014;114(3):480–492. doi:10.1161/CIRCRESAHA.114.302113
124. Hursitoglu O, Kurutas EB, Strawbridge R, et al. Serum NOX1 and Raftlin as new potential biomarkers of Major Depressive Disorder: a study in treatment-naive first episode patients. *Prog Neuro Psychopharmacol Biol Psychiatry.* 2023;121:110670. doi:10.1016/j.pnpbp.2022.110670
125. Liu T, Zhong S, Liao X, et al. A Meta-Analysis of Oxidative Stress Markers in Depression. *PLoS One.* 2015;10(10):e0138904. doi:10.1371/journal.pone.0138904
126. Tran N, Mills EL. Redox regulation of macrophages. *Redox Biol.* 2024;72:103123. doi:10.1016/j.redox.2024.103123
127. Li Q, Harraz MM, Zhou W, et al. Nox2 and Rac1 Regulate H₂O₂-Dependent Recruitment of TRAF6 to Endosomal Interleukin-1 Receptor Complexes. *Mol Cell Biol.* 2006;26(1):140–154. doi:10.1128/MCB.26.1.140-154.2006
128. Li Q, Engelhardt JF. Interleukin-1 β Induction of NF κ B Is Partially Regulated by H₂O₂-mediated Activation of NF κ B-inducing Kinase. *J Biol Chem.* 2006;281(3):1495–1505. doi:10.1074/jbc.M511153200
129. Ives A, Nomura J, Martinon F, et al. Xanthine oxidoreductase regulates macrophage IL1 β secretion upon NLRP3 inflammasome activation. *Nat Commun.* 2015;6(1):6555. doi:10.1038/ncomms7555
130. Nomura J, Kobayashi T, So A, Busso N. Febuxostat, a Xanthine Oxidoreductase Inhibitor, Decreases NLRP3-dependent Inflammation in Macrophages by Activating the Purine Salvage Pathway and Restoring Cellular Bioenergetics. *Sci Rep.* 2019;9(1):17314. doi:10.1038/s41598-019-53965-x

131. Sánchez-Rodríguez R, Munari F, Angioni R, et al. Targeting monoamine oxidase to dampen NLRP3 inflammasome activation in inflammation. *Cell Mol Immunol.* 2021;18(5):1311–1313. doi:10.1038/s41423-020-0441-8
132. Mullen L, Mengozzi M, Hanschmann EM, Alberts B, Ghezzi P. How the redox state regulates immunity. *Free Radic Biol Med.* 2020;157:3–14. doi:10.1016/j.freeradbiomed.2019.12.022
133. Billingham LK, Stoolman JS, Vasan K, et al. Mitochondrial electron transport chain is necessary for NLRP3 inflammasome activation. *Nat Immunol.* 2022;23(5):692–704. doi:10.1038/s41590-022-01185-3
134. Gabelloni ML, Sabbione F, Jancic C, et al. NADPH oxidase derived reactive oxygen species are involved in human neutrophil IL-1 β secretion but not in inflammasome activation. *Eur J Immunol.* 2013;43(12):3324–3335. doi:10.1002/eji.201243089
135. Devant P, Boršič E, Ngwa EM, et al. Gasdermin D pore-forming activity is redox-sensitive. *Cell Rep.* 2023;42(1):112008. doi:10.1016/j.celrep.2023.112008
136. Du G, Healy LB, David L, et al. ROS-dependent S-palmitoylation activates cleaved and intact gasdermin D. *Nature.* 2024;630(8016):437–446. doi:10.1038/s41586-024-07373-5
137. Humphries F, Shmuel-Galia L, Ketelut-Carneiro N, et al. Succination inactivates gasdermin D and blocks pyroptosis. *Science.* 2020;369(6511):1633–1637. doi:10.1126/science.abb9818
138. Mazur-Bialy AI, Pochec E, Plytycz B. Immunomodulatory effect of riboflavin deficiency and enrichment - reversible pathological response versus silencing of inflammatory activation. *J Physiol Pharmacol.* 2015;66(6):793–802.
139. Hochgerner H, Zeisel A, Lönnerberg P, Linnarsson S. Conserved properties of dentate gyrus neurogenesis across postnatal development revealed by single-cell RNA sequencing. *Nat Neurosci.* 2018;21(2):290–299. doi:10.1038/s41593-017-0056-2
140. Burm SM, Zuidervijk-Sick EA, Jong TAEJ, et al. Inflammasome-Induced IL-1 β Secretion in Microglia Is Characterized by Delayed Kinetics and Is Only Partially Dependent on Inflammatory Caspases. *J Neurosci.* 2015;35(2):678–687. doi:10.1523/JNEUROSCI.2510-14.2015
141. Sudo K, Takezawa Y, Kohsaka S, Nakajima K. Involvement of nitric oxide in the induction of interleukin-1 beta in microglia. *Brain Res.* 2015;1625:121–134. doi:10.1016/j.brainres.2015.08.030
142. Choi SH, Aid S, Kim HW, Jackson SH, Bosetti F. Inhibition of NADPH oxidase promotes alternative and anti-inflammatory microglial activation during neuroinflammation: NADPH oxidase regulates microglia phenotype. *Journal of Neurochemistry.* 2012;120(2):292–301. doi:10.1111/j.1471-4159.2011.07572.x
143. Wang S, Chu CH, Stewart T, et al. α -Synuclein, a chemoattractant, directs microglial migration via H₂O₂-dependent Lyn phosphorylation. *Proc Natl Acad Sci USA.* 2015;112(15). doi:10.1073/pnas.1417883112
144. Garrison AM, Parrott JM, Tuñon A, Delgado J, Redus L, O'Connor JC. Kynurenine pathway metabolic balance influences microglia activity: targeting kynurenine monooxygenase to dampen neuroinflammation. *Psychoneuroendocrinology.* 2018;94:1–10. doi:10.1016/j.psyneuen.2018.04.019
145. Honorat JA, Kinoshita M, Okuno T, et al. Xanthine Oxidase Mediates Axonal and Myelin Loss in a Murine Model of Multiple Sclerosis. *PLoS One.* 2013;8(8):e71329. doi:10.1371/journal.pone.0071329
146. Zhang Q, Lan Y, Fei HX, et al. Allopurinol protects against ischemic insults in a mouse model of cortical microinfarction. *Brain Res.* 2015;1622:361–367. doi:10.1016/j.brainres.2015.07.010
147. Mallajosyula JK, Kaur D, Chinta SJ, et al. MAO-B Elevation in Mouse Brain Astrocytes Results in Parkinson's Pathology. *PLoS One.* 2008;3(2):e1616. doi:10.1371/journal.pone.0001616
148. Zhang M, Chen H, Zhang W, et al. Biomimetic Remodeling of Microglial Riboflavin Metabolism Ameliorates Cognitive Impairment by Modulating Neuroinflammation. *Adv Sci.* 2023;10(12):2300180. doi:10.1002/adv.202300180
149. Pan LA, Naviaux JC, Wang L, et al. Metabolic features of treatment-refractory major depressive disorder with suicidal ideation. *Transl Psychiatry.* 2023;13(1):393. doi:10.1038/s41398-023-02696-9
150. Anandam KY, Alwan OA, Subramanian VS, Srinivasan P, Kapadia R, Said HM. Effect of the proinflammatory cytokine TNF- α on intestinal riboflavin uptake: inhibition mediated via transcriptional mechanism(s). *Am J Physiol Cell Physiol.* 2018;315(5):C653–C663. doi:10.1152/ajpcell.00295.2018
151. Naviaux RK, Naviaux JC, Li K, et al. Metabolic features of chronic fatigue syndrome. *Proc Natl Acad Sci USA.* 2016;113(37):E5472–E5480. doi:10.1073/pnas.1607571113
152. Beach RS, Mantero-Atienza E, Shor-Posner G, et al. Specific nutrient abnormalities in asymptomatic HIV-1 infection. *Aids.* 1992;6(7):701–708. doi:10.1097/00002030-199207000-00013
153. Fernandez-Banares F, Abad-Lacruz A, Xiol X, et al. Vitamin status in patients with inflammatory bowel disease. *Am J Gastroenterol.* 1989;84(7):744–748.
154. Iwakawa H, Fukui T, Fukuwatari T, et al. Blood concentrations and renal clearance of water-soluble vitamins in outpatients with ulcerative colitis. *biom rep.* 2019. doi:10.3892/br.2019.1191
155. Gariballa S, Forster S, Powers H. Riboflavin Status in Acutely Ill Patients and Response to Dietary Supplements. *JPEN J Parenter Enteral Nutr.* 2009;33(6):656–661. doi:10.1177/0148607109336602
156. Gray A, McMillan DC, Wilson C, Williamson C, O'Reilly SJ, Talwar D. The relationship between plasma and red cell concentrations of vitamins thiamine diphosphate, flavin adenine dinucleotide and pyridoxal 5-phosphate following elective knee arthroplasty. *Clin Nutr.* 2004;23(5):1080–1083. doi:10.1016/j.clnu.2004.01.013
157. Berk M. Biomarkers in psychiatric disorders: status quo, impediments and facilitators. *World Psychiatry.* 2023;22(2):174–176. doi:10.1002/wps.21071
158. Ji Y, Biernacka JM, Hebbring S, et al. Pharmacogenomics of selective serotonin reuptake inhibitor treatment for major depressive disorder: genome-wide associations and functional genomics. *Pharmacogenomics J.* 2013;13(5):456–463. doi:10.1038/tpj.2012.32
159. Westlund KN, Denney RM, Kochersperger LM, Rose RM, Abell CW. Distinct Monoamine Oxidase A and B Populations in Primate Brain. *Science.* 1985;230(4722):181–183. doi:10.1126/science.3875898
160. Karve A, Jagtiani S, Chitnis K. Evaluation of effect of allopurinol and febuxostat in behavioral model of depression in mice. *Indian J Pharmacol.* 2013;45(3):244. doi:10.4103/0253-7613.111922
161. Shan X, Ji Z, Wang B, et al. Riboflavin kinase binds and activates inducible nitric oxide synthase to reprogram macrophage polarization. *Redox Biol.* 2024;78:103413. doi:10.1016/j.redox.2024.103413

162. Yazdanpanah B, Wiegmann K, Tchikov V, et al. Riboflavin kinase couples TNF receptor 1 to NADPH oxidase. *Nature*. 2009;460(7259):1159–1163. doi:10.1038/nature08206
163. Park KJ, Lee CH, Kim A, Jeong KJ, Kim CH, Kim YS. Death Receptors 4 and 5 Activate Nox1 NADPH Oxidase through Riboflavin Kinase to Induce Reactive Oxygen Species-mediated Apoptotic Cell Death. *J Biol Chem*. 2012;287(5):3313–3325. doi:10.1074/jbc.M111.309021
164. Schramm M, Wiegmann K, Schramm S, et al. Riboflavin (vitamin B₂) deficiency impairs NADPH oxidase 2 (Nox2) priming and defense against *Listeria monocytogenes*: immunity to infection. *Eur J Immunol*. 2014;44(3):728–741. doi:10.1002/eji.201343940
165. Cao Y, Luo F, Peng J, Fang Z, Liu Q, Zhou S. KMT2B-dependent RfK transcription activates the TNF- α /NOX2 pathway and enhances ferroptosis caused by myocardial ischemia-reperfusion. *J Mol Cell Cardiol*. 2022;173:75–91. doi:10.1016/j.yjmcc.2022.09.003
166. Wu Y, Li S, Wang W, Zhang D. Associations of dietary B vitamins intakes with depression in adults. *Int J Vitamin Nutr Res*. 2023;93(2):142–153. doi:10.1024/0300-9831/a000720
167. Rouhani P, Amoushahi M, Keshteli AH, et al. Dietary riboflavin intake in relation to psychological disorders in Iranian adults: an observational study. *Sci Rep*. 2023;13(1):5152. doi:10.1038/s41598-023-32309-w
168. Ashoori M, Saedisomeolia A. Riboflavin (vitamin B₂) and oxidative stress: a review. *Br J Nutr*. 2014;111(11):1985–1991. doi:10.1017/S0007114514000178
169. Ahn H, Lee GS. Riboflavin, vitamin B₂, attenuates NLRP3, NLRC4, AIM2, and non-canonical inflammasomes by the inhibition of caspase-1 activity. *Sci Rep*. 2020;10(1):19091. doi:10.1038/s41598-020-76251-7
170. Zempleni J, Suttie JW, Gregory JF, Stover PJ. Chapter 6 (Vitamin B₂). In: *Handbook of Vitamins*. Fifth edition ed. Taylor & Francis; 2014:191–267.
171. Appelman B, Charlton BT, Goulding RP, et al. Muscle abnormalities worsen after post-exertional malaise in long COVID. *Nat Commun*. 2024;15(1):17. doi:10.1038/s41467-023-44432-3
172. Luo X, Zhou Y, Yuan S, Chen X, Zhang B. The changes in metabolomics profile induced by intermittent theta burst stimulation in major depressive disorder: an exploratory study. *BMC Psychiatry*. 2023;23(1):550. doi:10.1186/s12888-023-05044-9
173. Nackenoff AG, Moussa-Tooks AB, McMeekin AM, Veenstra-VanderWeele J, Blakely RD. Essential Contributions of Serotonin Transporter Inhibition to the Acute and Chronic Actions of Fluoxetine and Citalopram in the SERT Met172 Mouse. *Neuropsychopharmacol*. 2016;41(7):1733–1741. doi:10.1038/npp.2015.335

Journal of Inflammation Research

Publish your work in this journal

The Journal of Inflammation Research is an international, peer-reviewed open-access journal that welcomes laboratory and clinical findings on the molecular basis, cell biology and pharmacology of inflammation including original research, reviews, symposium reports, hypothesis formation and commentaries on: acute/chronic inflammation; mediators of inflammation; cellular processes; molecular mechanisms; pharmacology and novel anti-inflammatory drugs; clinical conditions involving inflammation. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/journal-of-inflammation-research-journal>

Dovepress
Taylor & Francis Group