

EDUCATIONAL ARTICLE

Long COVID, POTS, CFS and MTHFR: Linked by Biochemistry and Nutrition

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ABSTRACT

The recent pandemic has energized research spotlighting chronic fatigue disorders. The similarities between Long COVID (LC) and Chronic Fatigue Syndrome (CFS), often accompanied by postural orthostatic tachycardia syndrome (POTS) are striking. Furthermore, the majority afflicted with LC and CFS may be those with methylenetetrahydrofolate reductase (MTHFR) polymorphisms, present in the majority of Americans and characterized by hypomethylation. Elevated homocysteine (Hcy) and depressed B9 and B12 may be links. Speculation about an association between these laboratory analytes and MTHFR abnormalities has been previously reported (Regland et al., 2015). The absence of a blood-brain barrier (BBB) in CNS circumventricular organs (CVOs) that control autonomic and neuroendocrine functions, problematic in LC, CFS, POTS, and MTHFR, is provocative. Diffusion of CNS Hcy is associated with brain fog, cognitive impairment, and dementia. This provides a distinct link between MTHFR variants and the fog of LC, CFS, and POTS. Small intestine bacterial overgrowth (SIBO), present in about 17% of Americans, is linked to POTS, mast cell activation syndrome (MCAS), and Ehlers Danlos syndrome (EDS). All exhibit histamine intolerance and female predominance. This may be due to hypomethylation and/or intestinal diamine oxidase (DAO) deficiency. Metabolism of monoamines and histamine requires methylation. Specific CNS nuclei in CVOs may also provide insight to the POTS paradox. The similar gut microbiomes of LC/CFS (and vitamin D deficiency) may via CVOs trigger an imbalance in glutamate/GABA neurotransmission that translates to neuroendocrine and baroreflex dysfunction. Homozygosity for the MTHFR 677T allele can facilitate hypermethylation via an alternative "rescue" riboflavin pathway triggered by significant Hcy increase. Hypermethylation predominates in Long Covid. The primary problem in these

syndromes is compromised mitochondrial function due to oxidative stress induced by an antioxidant shortfall. Victims are also frequently deficient in 25(OH)D3 (the storage form of vitamin D), magnesium, and B vitamins, consumed by the persistent chronic inflammatory state. Estrogen increases histamine, norepinephrine, and bradykinin (BKN), which may in part explain the brain fog and its predilection for females.

ADH = Antidiuretic hormone (vasopressin)

ARBs = Angiotensin receptor blockers

BBB = Blood brain barrier

BKN = Bradykinin

CFS = Chronic Fatigue Syndrome

COMT = Catechol-O-methyltransferase

CRH = Corticotropin-releasing hormone

CVOs = Circumventricular organs

DAO = Diamine oxidase

EDS = Ehlers Danlos syndrome

FB = Fibromyalgia

Glu = Glutamate

GnRH = Gonadotropin releasing hormone

Hcy = Homocysteine

HNMT = Histamine N-methyltransferase

HPA = Hypothalamus-pituitary-adrenal

LC = Long COVID

MCAS = Mast cell activation syndrome

MTHFR = Methylene tetrahydrofolate reductase
P5P = Pyridoxal-5-phosphate
POTS = Postural orthostatic tachycardia syndrome
PVN = Paraventricular nucleus
RLS = Restless legs syndrome
SAME = S-adenosylmethionine
SIBO = Small intestine bacterial overgrowth

INTRODUCTION

COVID-19 has unleashed a tsunami of research that has been further energized by the persistent and debilitating aftermath of Long COVID. Explaining the gender reversal from male preponderance in COVID-19 to female in LC is challenging and covering the range of symptoms intimidating. Although there have been a few hypotheses incorporating autoantibodies, nutrient deficiencies, and reactivation of EBV, resolution of the paradoxes remains frustratingly out of reach. Recent research has revealed that the BBB is not all-inclusive and that some areas of the CNS are not so protected. These are called circumventricular organs (CVOs). Other research has revealed a close association between Long Covid and Hcy levels, often elevated in those with two very common enzymatic polymorphisms for MTHFR (Ponti et al., 2021). Most with these polymorphisms are unaware. But serum Hcy may play a large role in Long COVID and may provide unique insight into its pathogenesis. Receptor polymorphisms, enzyme polymorphisms, and the impact of methylation on suppressor and promoter genes, create a maze. A hypothesis incorporating the gut microbiome, gender predilection, and differential methylation is presented.

HYPOTHESIS

The autonomic and neuroendocrine symptoms of LC, POTS, and CFS appear to be primarily determined by CNS nuclei located in areas lacking a BBB. Similar LC and CFS gut microbiomes incriminate low fiber diets that dictate low bacterial diversity. Dietary glutamate (Glu), an excitatory neurotransmitter, permeates CVOs and overwhelms inhibitory GABA neurotransmission, impeding release of specific hypothalamic hormones, including those causing low cortisol, low aldosterone, and low ADH with subsequent hypovolemia. The hypovolemia triggers an increase in norepinephrine. The baroreflex is also compromised. POTS is often associated with MCAS, EDS,

and histamine intolerance. Metabolism of histamine requires methylation and intestinal bacteria are primarily responsible for this. All one carbon metabolism (methylation) reactions occur within mitochondria. Both hypomethylation (COVID-19, POTS, 1298C/677T, FM, and CFS) and hypermethylation (LC, 677TT) increase Hcy. The oxidative stress induced by Hcy due to 1) gut microbiome lacking diversity, 2) compromised methylation (genetic or epigenetic), 3) chronic inflammation elicited by residual spike protein S in LC, or 4) persistent SARS-CoV-2 induced T cell lymphopenia reactivating dormant viruses all conspire to overwhelm mitochondria lacking an antioxidant shield. Estrogen and BKN (and histamine) enhance endothelial permeability and may play prominent supporting roles.

POSTURAL ORTHOSTATIC TACHYCARDIA SYNDROME

POTS represents a complex mixture of genetic and epigenetic inputs. It generally exhibits an 80%-90% female predominance, afflicts those 15-45, and is typically precipitated by a preceding viral infection (Fedorowski, 2018). In some POTS is felt due to a dysfunctional hypothalamus-pituitary-adrenal (HPA) axis that comes with a paradox – low renin and low aldosterone in the face of low blood volume (POTS paradox). This was originally reported in POTS with CFS and later in POTS with LC (Chadda et al., 2022). In addition to low renin and aldosterone CFS patients have lower levels of oxytocin, antidiuretic hormone (ADH), and corticotropin-releasing hormone (CRH) => less ACTH => less aldosterone, less cortisol in combination with less ADH => hypovolemia with reflexive increase in norepinephrine (Goldstein, 1996). POTS has often been partitioned into subsets, e.g., neuropathic, hyperadrenergic, low flow, high flow, and these can overlap. A dysfunctional HPA axis is felt causative for neuropathic POTS. Most POTS exhibit hypovolemia and low flow. Those with low flow POTS often report low plasma renin activity and elevated Ang II, resulting in vasoconstriction (Stewart et al., 2006). Cortisol and aldosterone are low in both LC (neuropathic) and CFS hyperadrenergic POTS (Okamoto et al., 2012). POTS is also directly related to Hcy levels, at least in the young (Li et al., 2018).

Many with low flow POTS have angiotensin II autoantibodies and/or beta adrenergic autoantibodies (Yu et al., 2018; Fedorowski et al., 2017; Badiudeen et al., 2019). In hyperadrenergic POTS autoantibodies accentuate expression (Wirth & Scheibenbogen, 2020). Angiotensin II antibodies have also been found in LC (Briquez et al., 2022). Angiotensin receptor blockers (ARBs) can block both autoantibodies (Yu et al., 2018; Barki-Harrington et al.,

2003). Elevated Angiotensin II, due to autoantibodies and well described in low flow POTS, might provide negative feedback inhibition of renin.

High flow POTS may be due to vasodilation instead of vasoconstriction. Histamine dilates blood vessels and increases their permeability. POTS in MCAS and EDS may represent high flow type.

Elevated Hcy is prevalent in the MTHFR variants and may provide one link between some forms of POTS and some of these polymorphisms. Some MTHFR variants that yield hypomethylation appear to be predisposing factors for MCAS, providing another link to POTS. Whether these links are cause or effect is not yet clear. However, methylation is required to degrade histamine, suggesting causation.

Like autoantibodies, Hcy can also activate AT1Rs (angiotensin II type 1 receptors) (Li et al., 2018). The Hcy induced redox imbalance can compromise aldosterone synthase (low aldosterone) and 11beta hydroxylase (low cortisol), both mitochondrial based (Häggström & Richfield, 2014).

Some MTHFR variants with significant hypomethylation are theoretically capable of developing either low flow POTS or high flow MCAS type POTS. The former could be caused by elevated norepinephrine, as catechol-O-methyltransferase (COMT) degrades monoamines (Hall et al., 2019). The latter could be caused by elevated histamine, degradation of which is dependent upon histamine N-methyltransferase (HNMT). Both require S-adenosylmethionine (SAmE) and both reactions occur in mitochondria (Malecki et al., 2022).

GUT MICROBIOME

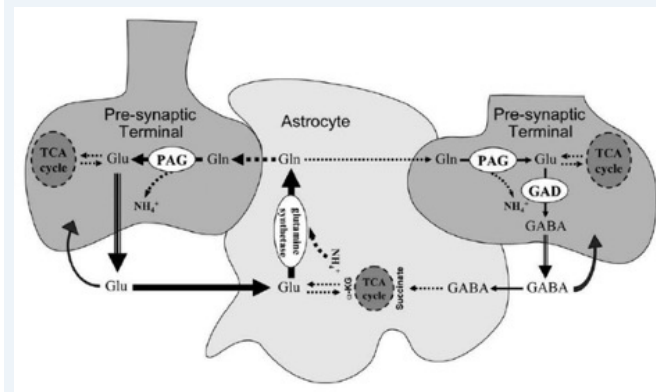
The neuropathic type of POTS seen in LC might be due to a CVO mediated Glu/GABA imbalance that leads to decreased ADH, oxytocin, and CRH. It is both a neuroendocrine and an autonomic disorder. LC and CFS are excellent models for the gut-brain axis. Gut microbiome studies of both LC (Yeoh et al., 2021) and CFS (König et al., 2021) report deficiencies in butyrate and GABA producing bacteria, e.g., Bacteroides (Strandwitz et al., 2018), Bifidobacterium dentium (Pokusaeva et al., 2017), and Lactobacillus brevis (Ko et al., 2013). Bacteroides not only drives gut microbiota diversity but also is one of only six known species that produces active B6 or pyridoxal-5-phosphate (P5P). B6 deficiency itself reduces the relative abundance of Bacteroides in the microbiota (Mayengbam et al., 2020). Without P5P glutathione cannot be synthesized from Hcy. A gut microbiome that lacks an abundance of histamine

degrading Bifidobacteria leaves allergic features unopposed (Dev et al., 2008). This may contribute to MCAS. Furthermore, GABA secreted by histaminergic neurons also downregulates histamine signaling (Yu et al., 2015). The endogenous pathways for the degradation of histamine require either methylation (histamine-N-methyltransferase) or intestinal DAO. Both require P5P. A suboptimal gut microbiome might compromise the function of DAO. All methyltransferase reactions require SAmE, P5P and magnesium. Many such reactions require functioning mitochondria.

LC, CFS, and by extension the oft accompanying POTS may share gut microbiome features that underscore the criticality of bacterial GABA synthesis to proper functioning of the HPA axis, accentuated by CVOs.

GABA receptor polymorphisms are linked with restless legs syndrome (RLS) (Jiménez-Jiménez et al., 2018). RLS is a subset of periodic limb movement disorder (PLMD) aka nocturnal myoclonus. This is considered to be a seizure disorder and is increased in LC (Weinstock et al., 2022), CFS (Civelek et al., 2014), and POTS (Dodson, et al., 2021). RLS is also associated with magnesium deficiency, especially in those on proton pump inhibitors. GABA and magnesium display anti-seizure properties. This further underscores the probable role of a Glu/GABA imbalance in the pathogenesis of POTS, LC, and CFS. RLS may also be linked to SIBO (Blum et al., 2019), further evidence of the gut-brain axis at work (see **Figure 1**). RLS is associated with MCAS and histamine intolerance. EDS is also linked to MCAS. RLS is increased in POTS. Hypomethylation, GABA/P5P/Mg⁺⁺ deficiency, and suboptimal gut microbiome might be at the root of all these syndromes.

Figure 1. Displayed are glutamatergic and GABAergic neurons. Note the magnitudes of the arrows (Riera et al., 2008). Note the need for TCA recycling of GABA within astrocytes.



CIRCUMVENTRICULAR ORGANS

Several recent articles have highlighted CVOs (Jammoul et al., 2023; Ganong, 2000). There are several areas within the brain that abut ventricles and have no BBB. Several of these CVOs contain nuclei that are quite relevant to the autonomic and neuroendocrine dysfunctions of LC. These CVOs are the pineal gland, the posterior pituitary a.k.a. neurohypophysis, the paraventricular nucleus (PVN) in the median eminence, the area postrema, the organum vasculosum of the lamina terminalis or supraoptic crest, and the subfornical organ (see **Figure 2**). The AP is part of the dorsal vagal complex, closely associated with baroreceptors and chemoreceptors, as well as cranial nerves VII and IX (see **Figure 3**).

Figure 2. Red structures designate CVOs (Bassi, 2015). The inferior area postrema is adjacent to the foramen of Magendie, a CSF passageway.

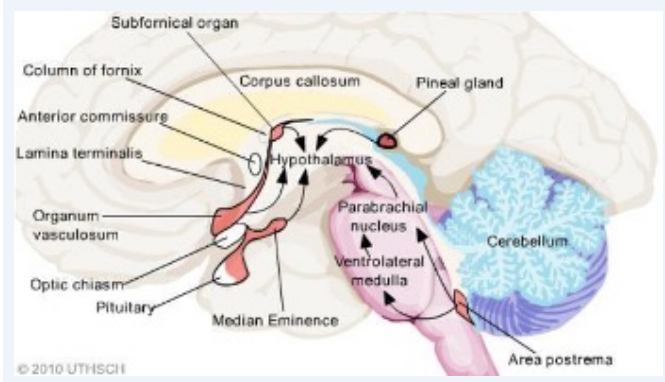
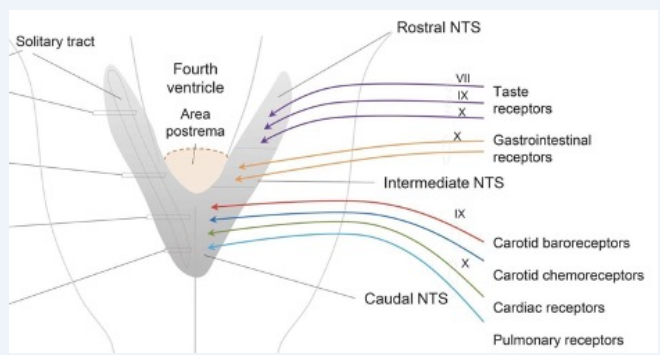


Figure 3. The AP, the dorsal motor nucleus of X, and the nucleus of the solitary tract (NTS) form the dorsal vagal complex (Cutsforth-Gregory & Benarroch, 2017).



CVOs enable diet induced excess of Glu to enter the CNS and create a Glu (excitatory)/GABA (inhibitory) imbalance to precipitate not only POTS but also symptoms that involve decreased oxytocin, vasopressin (ADH), CRH for ACTH, and gonadotropin releasing hormone (GnRH for LH, FSH).

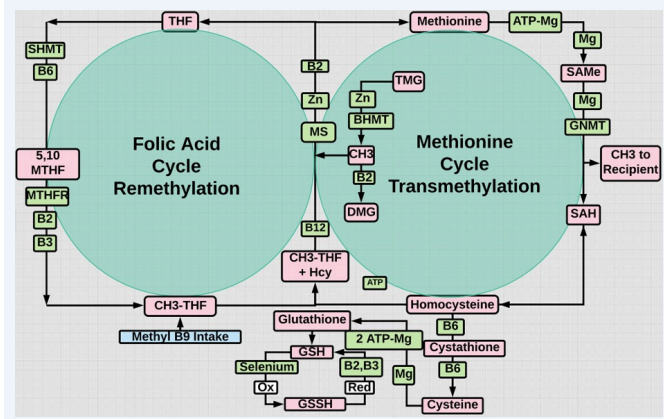
CVOs can conveniently explain the POTS paradox in the hypovolemic low flow subtype (majority). The PVN normally inhibits the baroreflex via tonic GABAergic inputs (Page et al., 2011). Just as sympathetic inhibition and parasympathetic activation respond to carotid and aortic stretch receptors to decrease BP, so sympathetic activation and parasympathetic inhibition are required to increase it upon standing. An imbalanced Glu (elevated)/GABA(depressed) impedes both, causing postural hypotension. GABAergic signals also positively dictate secretion of neurohypophyseal hormones, including ADH (Haam et al., 2012), oxytocin (Lee et al., 2015), CRH (Kakizawa et al., 2016) and GnRH (Moore et al., 2018). On the other hand GABA inhibits the secretion of TRH (Wiens & Trudeau, 2006)

CFS patients have experienced relief from desmopressin (Miwa, 2017) and CRH (Pereira et al., 2021). POTS and CFS patients have benefited from exogenous CRH and ADH (Coffin et al., 2012). Oxytocin in CFS increased stamina, decreased pain, improved cognitive function, decreased FM pain, anxiety, and depression (Diep et al., 2022). Some LC patients have experienced relief with oxytocin (Goldstein, 2012). Many POTS patients claim heat intolerance and this may be mediated by unopposed Glu (Amir, 1990).

MTHFR AND HOMOCYSTEINE

Polymorphisms in the MTHFR gene markedly compromise conversion of 5,10 -methylene tetrahydrofolate to 5-methyl tetrahydrofolate. The 1298C/677T (compound heterozygous) and 677TT MTHFR variants in order pose escalating bottlenecks, 35% and 70% respectively, in recycling Hcy to methionine (Frosst et al., 1995). MTHFR is critical to methylation. A fully functional MTHFR with the assistance of B2, B6, and B12 (methylcobalamin) helps methylate Hcy, recycling methionine (see **Figure 4**). If the active form of B6 (P5P) is insufficient, then glutathione, the master antioxidant, will not be produced with additional buildup in Hcy. ROS, induced by Hcy, will increase and mitochondrial function is compromised, especially in endothelial cells.

Figure 4. Recycling of Hcy in the methionine cycle is enabled by methyl THF, otherwise compromised by MTHFR gene variants. Activated B2, B6, and B12 are required in the folate or re-methylation cycle. Pyridoxal phosphate (P5P) is B6 (Al-Sadeq & Nasrallah, 2020). B2 is also required to recycle oxidized glutathione back to its reduced antioxidant state.



The MTHFR 677T and 1298C alleles (variants) vary ethnically, but are present in about 33% and 38% of Caucasians respectively (Nefic et al., 2018). However, according to the CDC, there are more people in the United States who have one or two copies of the MTHFR C677T variant than people who do not. Perhaps 10-20% of the American population have 677TT. So this is an exceedingly common polymorphism with incidence that roughly somewhat reflects the incidence of LC.

Hcy is a strong marker for LC (Ponti et al., 2021), MTHFR variants (Carpenè et al., 2022), and for CFS (Regland et al., 1997). Both histamine and Hcy require methylation for their metabolism, which suggests that hypomethylation may cause histamine intolerance in MCAS and EDS. High Hcy may be a good marker for MTHFR variants and low folate a good inverse marker for Hcy (Bottiglieri et al., 2000). Hcy is a strong marker for thrombosis. CNS Hcy is associated with brain fog that may be mediated by CVOs (Oner et al., 2023).

Compound heterozygous MTHFR gene variants (677T/1298C) characteristically exhibit hypomethylation (Ren et al., 2018). In a recent review compared to controls, patients that experienced mild/moderate COVID-19 or LC exhibited hypomethylation and hypermethylation respectively (Nikesjö et al., 2022). This reflects the hypomethylation disposition of MTHFR 1298C/677CT (slightly elevated Hcy) and the hypermethylation disposition of MTHFR 677TT (more pronounced elevation

of Hcy). Hcy associated with the 677TT variant appears to trigger hypermethylation via an alternative B2 dependent “rescue” pathway (Fryar-Williams, 2016).

Hypermethylation of the promoter region for the MTHFR gene silences it, further compromising transmethylation (Shaker et al., 2021). Those with 677TT usually need higher doses of 5-methyltetrahydrofolate (methylfolate). Methylation status in the body can be determined by measuring whole blood histamine, as histamine and methylation are inversely related. Low blood histamine levels indicate overmethylation; high levels indicate undermethylation (Walsh, 2012)⁶¹. Histamine is predominantly metabolized via methylation and is upregulated by estrogen (Bonds & Midoro-Horiuti, 2013).

This suggests that mild to moderate Covid 19 might include many with hypomethylated 1298C/677CT and LC might include many with hypermethylated 677TT. Epigenetic hypermethylation can persist for one year after hospitalization (Balnis et al., 2022). The hypermethylated have higher Hcy levels, which portend more serious COVID-19 and LC (Kalan Sari et al., 2023). Indeed those without the MTHFR variants might comprise a large portion of the asymptomatic.

Hcy may be the primary common denominator linking LC, CFS, POTS, and MTHFR variants. Hcy is increased in LC, CFS, and MTHFR 1298C/677T and 677TT. Some with low flow POTS exhibit oxidation of the vasodilator NO with microvascular endothelial dysfunction (Medow et al., 2005). Hcy promotes oxidant injury to endothelial cells and microthrombosis, prominent features of LC and CFS. Hcy is directly related to oxidative stress and inversely reflects B vitamin sufficiency, especially that of B6. Two steps in the metabolism of Hcy to glutathione, the master antioxidant, require B6 (see **Figure 4**).

A suboptimal diet and gut microbiome (low microbiota diversity) might compromise absorption of exogenous antioxidants, enhancing the pro-oxidative damage wreaked by Hcy. Many ROS producing mitochondria are shut down to enable cell survival and avoid lysis. Most endogenous antioxidants require methylation and SAME, the universal methyl donor, i.e., cofactor for all methyltransferases. All SAME methylations require ATP and occur in mitochondria (Malecki et al., 2022; Rhein et al., 2014). Mg⁺⁺ is a required chelate for all reactions requiring ATP and a required cofactor for all SAME reactions. Methylation is also critical to the degradation of neurotransmitters and hormones, including serotonin, dopamine, epinephrine, norepinephrine and melatonin. A suboptimal gut microbiome in combination with mitochondrial dysfunction

retards synthesis of 1,25(OH)₂D₃.

Ultimately LC, CFS, and low flow POTS seem to stem from antioxidant insufficiency (redox imbalance) and mitochondrial dysfunction. Elevated Hcy of endogenous origin, whether genetic (MTHFR) or epigenetic (SAmE related), determined in part by nutrition, assisted by CVO/BKN induced permeability, and characterized by altered 1) mitochondrial function, 2) neurotransmission, 3) enzyme activity, may represent the final common pathway. Elevated histamine and BKN in females also appear to be major players in the hypomethylated. MTHFR variants with differential methylation may be more susceptible to some, e.g., CFS (de Vega et al., 2014), or all of these.

As a corollary, chronic inflammation and age increase differentially methylated DNA and subsequent cancer risk, especially breast and colorectal cancers (Benelli et al., 2021). An elevated Hcy is associated with cardiovascular disease, depression, dementia, AD, and cancer (McKinney et al., 2019).

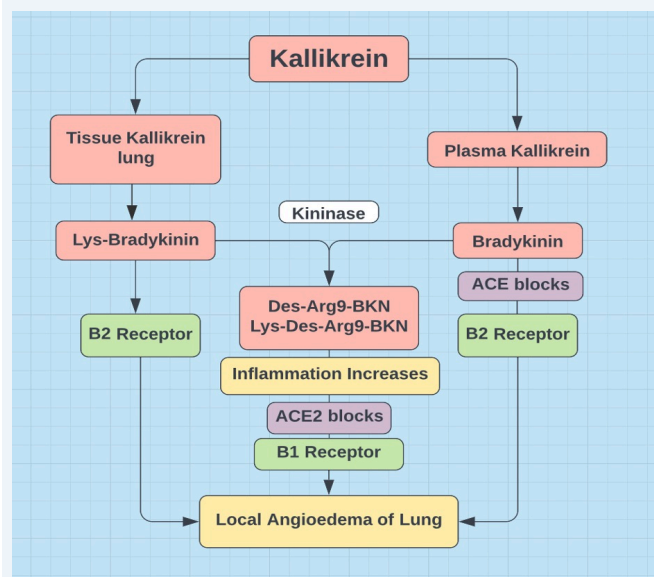
FEMALE PREPONDERANCE

Females exhibit more robust T cell activation than males (Takahashi et al., 2020). Females have higher levels of type I IFN alpha and beta (Pujantell & Altfeld, 2022). T cell production of IFN gamma (IFN type II) is triggered by IFN alpha and beta. It is a secondary release, STAT dependent IFN (Chambers, 2022). T cell population decreases with age in males but not females. T cell production of IFN-γ persists in females (Goetzl et al., 2010). Increased autoimmune disease in females may be due to their enhanced innate immunity and production of pleiotropic IFN-γ, secreted by CD4⁺ and CD8⁺ T cells. When combined with chronic low grade inflammation, this proinflammatory pleiotropic cytokine might switch from self recognition to loss of self-recognition or autoimmune status (De Benedetti et al., 2021).

IFN-γ produced by these CD4⁺ and CD8⁺ T cells also induces hepatic synthesis of C1 esterase inhibitors (C1INH). Loss of complement inhibition initiates the classic complement pathway, which cross talks with the kallikrein kinin system (KKS). BKN is then also upregulated (Bossi, 2011). Lymphopenia (primarily of T cells) is associated with persistence of COVID-19 symptoms. CD147 (basigin) receptors but not ACE2 receptors are found on peripheral blood mononuclear cells (Kuklina, 2022). This receptor when bound to the CD147 epitope on the spike protein S (Chambers, 2021) may trigger the complement cascade and mediate lymphopenia. Any viral infection that challenges T lymphocytes increases BKN.

Estrogen may play a role in low flow POTS by decreasing renin levels, decreasing ACE activity and thereby increasing BKN, decreasing AT1 receptor density and decreasing aldosterone (Ma et al., 2021). In addition estrogen appears to lower cortisol (Herrera et al., 2017) and ACE2, at least in the lungs (Stelzig et al., 2020). Perhaps lower pulmonary ACE2 is Covid 19 protective, but downregulated ACE2 may be associated with higher systemic levels of des-Arg9-bradykinin (see **Figure 5**). Des-arg-BKN appears to be a prominent kinin in COVID-19 (Mendes et al., 2022).

Figure 5. ACE2 degrades des-argBKN (BK1R) and ACE degrades BKN (BK2R) => ACE and ACE2 are neuroprotective (van de Veerdonk, 2020).



Additionally vascular dilation and leakage induced by BKN might aggravate hypovolemia in high flow POTS, e.g., POTS in MCAS. A preceding viral respiratory infection markedly upregulates BKN (Stewart et al., 2008). Down regulation of ACE (ACE degrades BKN) by estrogen combined with a viral infection can supercharge BKN. BKN indirectly inhibits renin to a greater than RAS directly inhibits renin (Kiowski et al., 1992). This might in part explain the decreased renin in predominantly female POTS and its paradox. An increased Ca/Mg might also impact this, as Mg⁺⁺ is required to synthesize cAMP and cGMP that mediate the release of renin from the juxtaglomerular apparatus (Persson, 2003).

Upregulation of BKN and histamine by estrogen in combination with hypomethylation (mild increase in

homocysteine) may be linked to the pathogenesis of high flow in female dominated POTS, SIBO, MCAS, and EDS.

A more significant increase in Hcy might elicit hypermethylation by an alternative “rescue” pathway (Fryar-Wiliams, 2016). The associated endothelial redox imbalance diminishes arterial sensitivity to norepinephrine, possibly driving a reflexive increase, as seen in CFS with hyperadrenergic POTS.

VITAMIN D AND OXIDATIVE STRESS IN MITOCHONDRIA

Men with the highest compared to lowest $1,25(\text{OH})_2\text{D}$ and activation ratios, i.e., active form/storage form ($1,25(\text{OH})_2\text{D}/25(\text{OH})\text{D}$), are more likely to possess butyrate-producing bacteria associated with favorable gut microbial health. Sun exposure may affect the storage form of vitamin D, but it exerts little influence on the active hormone (Thomas et al., 2020). However, endothelium, targeted by Hcy, may benefit from D3 alone (Gibson et al., 2015).

Mitochondrial health is especially important in maintaining optimal levels of active vitamin D. Oxidative stress (ROS due to inflammation, smoking, toxins, ...) compromises mitochondrial oxidative phosphorylation. C-reactive protein reflects oxidative stress (Moreira-Rosário et al., 2021) and is a marker for LC, CFS, and vitamin D deficiency. Mitochondrial CYP27B1 is the only enzyme (requires Mg^{++}) that hydroxylates carbon number one in cholecalciferol, activating $25(\text{OH})\text{D}_3$ (Bikle, 2014; Warren et al., 2021).

Once chronic inflammation becomes entrenched and mitochondria oxidatively stressed (less CYP27B1) exogenous $1,25(\text{OH})_2\text{D}_3$ is needed.

Most with POTS have $25(\text{OH})\text{D}_3$ levels below 20 nm/mL (Ashangari & Suleman, 2015). There is a seasonal variation to the gut microbiome, as there is to $25(\text{OH})\text{D}_3$. Viral respiratory illness increases in the winter and induces robust depletion of the gut microbiome (Yildiz et al., 2018). Although the gut microbiota in females is larger and more diverse than that in males, the greater incidence of URIs in females (Groeneveld et al., 2020) with concomitant microbiota depletion may explain the female gender disparity in these syndromes. A suboptimal diet, less sun exposure, and greater risk of a viral respiratory illness may conspire to produce this suboptimal gut microbiome (Al-Khaldy et al., 2023; Waterhouse et al., 2019; Singh et al., 2020), enhancing susceptibility to not only LC and CFS but also post viral fatigue syndrome in general, POTS (Blitshteyn, 2017), SIBO, MCAS, EDS, and some MTHFR variants (Okamoto et al., 2012).

ANTIOXIDANTS IN POST VIRAL FATIGUE SYNDROME

The symptom list for CFS is long and recapitulates many of the symptoms of LC, FM, CMV, and EBV. Indeed these are all probably best classified under the umbrella of post viral fatigue syndrome. There are numerous articles and at least one book that extoll the value of antioxidants aka mitochondrial optimizers or enhancers in the treatment of CFS⁹⁸. Many that develop CFS can retrospectively recall an episode of viral illness that preceded the onset of initial symptoms. Like those with POTS, many with CFS and LC are vitamin D deficient.

Low gut microbiota diversity is associated with CFS, LC, and deficiencies of vitamin D (Thomas et al., 2020), magnesium, zinc, selenium, and other micronutrients. Vitamin D deficiency is often accompanied by magnesium deficiency, as synthesis of the active form of vitamin D from cholesterol requires the cofactor Mg^{++} in half a dozen enzymatic reactions. B2 and Mg^{++} are required cofactors for the synthesis of 7-dehydrocholesterol from which D3 and cholesterol are produced.

Synthesis of aldosterone requires aldosterone synthase and synthesis of cortisol requires 11beta hydroxylase. These two enzymes are part of the CYP450 system, all of which require Mg^{++} . Furthermore these two reactions occur in the mitochondria, i.e., they also require Mg^{++} chelated to ATP. So magnesium deficiency might also contribute to the low aldosterone and cortisol in the POTS paradox. If oxidative stress is sufficient and antioxidants are not, mitochondria are shut down to avoid cell lysis and ATP is down regulated, limiting synthesis of aldosterone and cortisol (Atanassova & Koeva, 2012). Females tend to be more magnesium deficient than males before age 50 (see **Figure 6**). An elevated Ca:Mg may also compromise synthesis of aldosterone and cortisol (Kiowski, 1992; Persson, 2003), as calcium and magnesium both compete for the same receptor, the calcium sensing receptor.

MAGNESIUM AND THE B VITAMINS

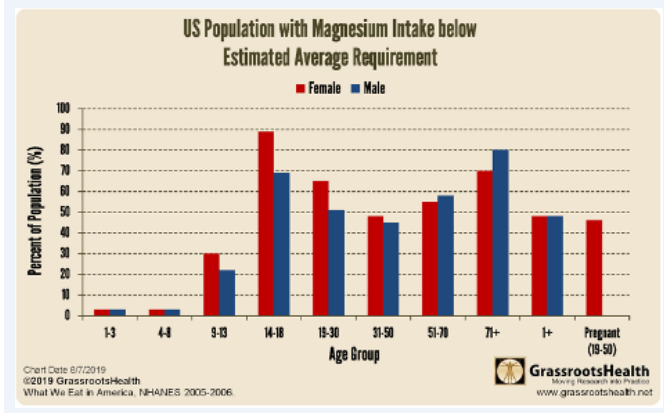
Magnesium is also required for the synthesis of all B vitamins, except biotin (B7). Mitochondrial function is dependent upon adequacy of all the B vitamins (Depeint et al., 2006). If mitochondrial function is marginal, production of the active form of vitamin D may be marginal. Mitochondrial CYP27B1 requires B2 and Mg^{++} as cofactors.

Alcohol intolerance is a primary complaint in LC (alcohol tastes different), CFS, and MCAS. Hepatic metabolism of alcohol yields acetaldehyde, but further degradation in the oxidatively stressed mitochondria is compromised with

symptoms ranging from headache to hangover, both acetaldehyde induced.

Thiamine (B1), riboflavin (B2), niacin (B3), pantothenate (B5), and pyridoxine (B6) require phosphorylation for activation. Cyanocobalamin (B12) and folate (B9) require methylation (S_{AM}e) for activation. Mg⁺⁺ is a required chelate for phosphorylation and a required cofactor for methylation. Hcy is highly influenced by B vitamin status (Azzini et al., 2020).

Figure 6. Females less than 50, the demographic representing the vast majority of individuals diagnosed with POTS, are more Mg⁺⁺ deficient than their male counterparts.



The last enzymatic steps to produce serotonin, dopamine, and GABA are decarboxylases that require active B6 (P5P), as a cofactor. Melatonin and glutathione synthesis also require B6. Norepinephrine synthesis requires Mg⁺⁺. A shortfall in magnesium or B6 compromises monoamine neurotransmitter synthesis and leads to depression, a symptom shared by LC, POTS, CFS, MCAS, and EDS.

Genetic (MTHFR) and/or epigenetic hypomethylation appears to link CFS, MCAS, POTS, and EDS. All syndromes predominantly affect females and all may in part be due to inability to methylate B9 and B12. Indeed P5P is required to synthesize P5P from pyridoxine. Histamine is degraded either via the methyltransferase pathway or via DAO. Both pathways require P5P. All methyltransferases require Mg⁺⁺ as a cofactor (Doctor's Data, Inc). Furthermore, estrogen upregulates histamine and vice versa (Bonds & Midoro-Horiuti, 2013). Histamine also upregulates NE.

In a clinical study of 33 POTS patients with GI symptoms 27 had SIBO, 9 had MCAS and 14 had EDS (Weinstock et al., 2018). SIBO has been linked to EDS (Uy et al., 2021) and is more common in females (Rao & Bhagatwala, 2019).

Gut dysbiosis, including SIBO, is associated with MCAS due to diamine oxidase (DAO) deficiency (Sánchez-Pérez et al., 2022).

Histamine is processed primarily in the intestines by methyltransferase or DAO. An April 2023 review suggested that MTHFR polymorphisms play a large role in EDS (Courseault et al., 2023).

High doses of pyridoxine are associated with peripheral neuropathy, whereas high doses of the active form, pyridoxal-5-phosphate, are not (Hadtstein & Vrolijk, 2021). Unfortunately a nutritious diet is becoming less available due to soil mineral depletion, enhancing the need for supplementation.

THOUGHTS ON THERAPY

Adding some fiber (prebiotic), e.g., sauerkraut, kimchi, with judicious supplementation, especially magnesium and D3, seems a prudent first step to cultivate good gut microbiota, e.g., bifidobacteria, lactobacillus. Probiotics or live bacteria in a pill also help in the search for the optimal gut microbiome. The heavy emphasis of dairy in the Western diet has resulted in an ever increasing Ca/Mg ratio. The 1977 NHANES (National Health and Nutrition Examination Survey) data indicated a ratio of 2.6. This ratio first exceeded 3.0 in the 2000 survey. When elevated, sustaining a 25(OH)D₃ level of 50 ng/mL (requires about 4000 IUs/day) becomes difficult, as these divalent cations compete for the same receptor. All post viral fatigue syndromes are due to ROS induced loss of mitochondria due to a shortage of onboard antioxidants. Furthermore, any antioxidant or mitochondrial shortfall impedes activation to 1,25(OH)₂D₃. Selenium, zinc, vitamin C, all fat soluble vitamins (A, D, E, K) are excellent antioxidants (Chambers, 2022).

Methylated forms of B9 and B12 are vital, if an MTHFR variant and/or elevated Hcy is discovered. Any tolerable (avoid laxative effect) increase in magnesium intake is especially recommended. If truly desperate, an ARB, under supervision, might be worth exploring.

The potential benefits of ARBs for LC include:

1. ARBs upregulate GABA, which displays anti-hypertension, anti-senescence, anti-diabetes, antioxidant, and anti-inflammatory properties (Sfera et al., 2022).
2. ARBs increase ACE, which degrades BKN, but block AT1Rs and hypertension induced dementia (Campbell et al., 1995)
3. ARBs alleviate POTS (Medow et al., 2005) and degrade BKN otherwise associated with AD (Singh et al., 2020).

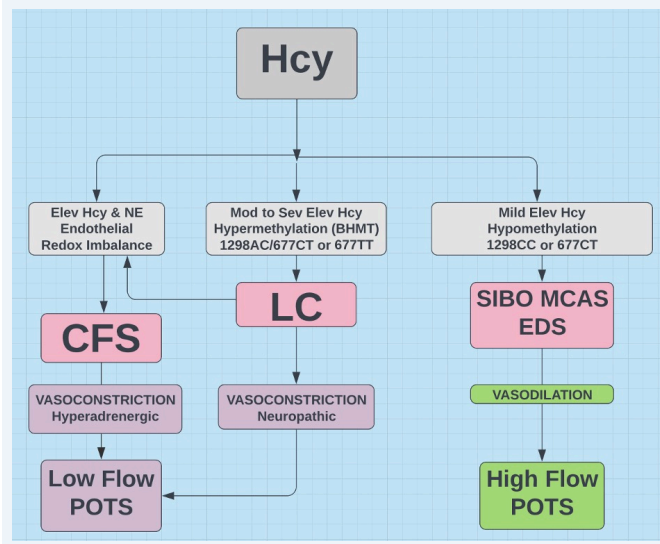
4. ARBs increase ACE2 (Zaheer et al., 2021) that degrades amyloid β -peptide ($A\beta$) in AD (Kehoe et al., 2016). The benefits of ACE2 in COVID-19 exceed its risks as a receptor for SARS-CoV-2 (Bastolla et al., 2022).
5. ARBs down regulate pro-inflammatory transforming growth factor-beta (TGF- β), increased in LC (Mahudpour et al., 2020), CFS (Yang et al., 2019), AD (Zhang et al., 2016), Marfan Syndrome, and EDS.
6. ARBs improve insulin sensitivity (Zhang et al., 2013) and are antidiabetic (Chang et al., 2014).
7. ARBs are neuroprotective (Villapol & Saavedra, 2015).

Indeed, a post mortem study of brains demonstrated more neuropathology in non-hypertensives, than in hypertensives on ARBs (Hoffman et al., 2009). Furthermore, angiotensin II-stimulating antihypertensives, e.g., ARBs, exhibited lower dementia risk than angiotensin II inhibiting antihypertensives, e.g., ACE inhibitors (van Dalen et al., 2021).

CONCLUSION

Three hypothetical conclusions involving the gut microbiome, female gender propensity, and transmethylation appear to link LC, POTS, CFS, and some MTHFR variants. Hcy appears to play a vital role (see **Figure 7**) and its elevation in some MTHFR variants suggests increased risk.

Figure 7. Proposed flow chart indicating hypothetical relationships for LC, CFS, SIBO, MCAS, EDS, POTS, and MTHFR with Hcy as common denominator.



- Lack of gut microbiota diversity and elevated C-reactive protein, indicative of chronic inflammation, are independent prognostic biomarkers for LC, CFS, and vitamin D deficiency (Moreira-Rosário et al., 2021). MTHFR mutations do not directly compromise gut microbiota diversity. The absence of the BBB in CVOs with nuclei that control baroreceptor function (area postrema and PVN) and neuroendocrine function (PVN), combined with increased Glu, histamine and decreased GABA producing bacteria, may in part explain pathogenesis of the myriad symptoms in these syndromes. Glu permeates CVOs and may overwhelm GABA neurotransmission. This suboptimal microbiome aggravates symptoms in those with the MTHFR 677T genotype (Regland et al., 2015; Ponti et al., 2021) and is seen in the vitamin D deficient (Thomas et al., 2020). Most with POTS have low 25(OH)D3 levels (Ashangari & Suoleman, 2015). Seasonal variation in the gut microbiome (Machado, 2023) may reflect the cold and flu season due to low vitamin D. These URIs cause and are caused by a sub optimal gut microbiome (Yildiz et al., 2018; Al-Khaldy et al., 2023; Waterhouse et al., 2019; Singh et al., 2020), increasing susceptibility to post viral fatigue syndrome, LC, CFS, POTS (Blitshteyn et al., 2017), and some MTHFR variants (Okamoto et al., 2012). Unfortunately once chronic inflammation becomes entrenched and mitochondria oxidatively stressed (less CYP27B1) exogenous 1,25(OH)₂D3 is needed (Warren et al., 2021). SIBO is an issue separate from gut microbiome diversity. Colon bacteria gain a foothold in the small bowel. This is associated with low volume high flow POTS seen in MCAS and EDS with histamine intolerance.

- Females in their reproductive years appear to be more susceptible to LC. This group is also more likely to be magnesium deficient (see figure 7), probably due to the Western diet, high in calcium and low in magnesium. Estrogen, a mild ACE inhibitor, promotes BKN, linked to brain fog, cognitive decline, and AD. Estrogen also upregulates IFN- γ , produced by CD4⁺ and CD8⁺ T cells. Decreased IFN- γ translates to decreased C1INH and activation of the classic complement pathway and the KKS. Respiratory viral infections, increased in females, induce additional release of BKN (Stewart et al., 2008)⁸². Estrogen induced upregulation of histamine (degradation requires transmethylation) enhances endothelial permeability and MCAS type low volume high flow POTS. The “post viral fatigue syndrome” in general may stem from a discriminating viral assault on T cells or from a URI induced depletion of gut microbiota. The loss of IFN- γ and the presence of estrogen potentiate chronic inflammation and endothelial permeability. Subsequent

chronic inflammation induced oxidative stress from Hcy, residual spike protein S, and reactivated viruses conspire to overwhelm those with insufficient onboard antioxidants. In the absence of critical cofactors synthesis of the master antioxidant glutathione from Hcy cannot proceed (see figure 5). This downregulates mitochondrial activity and upregulates fatigue (Wood et al., 2021). This also compromises the synthesis of 1,25(OH)₂D₃, the active form of vitamin D, and with it immune function.

- In the presented hypothesis protein methylation plays a prominent role. It is at the center of the MTHFR gene variants. It manifests in LC (Nikesjö et al., 2022) and POTS (Li et al., 2018) high flow type. MTHFR status is easily available via 23andme or a blood test for Hcy. However, given the prevalence of the MTHFR variants in the general population and from which the laboratory reference values for Hcy are determined, any value near the upper limit of normal may be cause for concern. Elevated Hcy carries many short term (thrombosis) and long-term (neurodegenerative disease) problems, best addressed in a timely manner. Mild elevation of Hcy is associated with hypomethylation. Significantly elevated Hcy associated with the 677TT variant appears to trigger hypermethylation via an alternative B2 dependent "rescue" pathway (Fryar-Williams, 2016). Another protein impacted by hypomethylation is histamine linked to SIBO. DNA can also be differentially methylated (hypo and hyper methylated regions) and is associated with chronic inflammation, cancer, and increasing age. Given the high frequency of this polymorphism in the general population and its potential devastating effects, everyone should be privy to their risks and those of their offspring, especially in the post Covid era.

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