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Phenylketonuria oxidative stress and energy dysregulation: Emerging pathophysiological elements provide interventional opportunity

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Abstract

Phenylalanine hydroxylase (PAH) deficient phenylketonuria (PKU) is rightfully considered the paradigm treatable metabolic disease. Dietary substrate restriction (i.e. phenylalanine (Phe) restriction) was applied >60 years ago and remains the primary PKU management means. The traditional model of PKU neuropathophysiology dictates blood Phe over-representation directs asymmetric blood:brain barrier amino acid transport through the LAT1 transporter with subsequent increased cerebral Phe concentration and low concentrations of tyrosine (Tyr), tryptophan (Trp), leucine (Leu), valine (Val), and isoleucine (Ile). Low Tyr and Trp concentrations generate secondary serotonergic and dopaminergic neurotransmitter paucities, widely attributed as drivers of PKU neurologic phenotypes. White matter disease, a central PKU characteristic, is ascribed to Phe-mediated tissue toxicity. Impaired cerebral protein synthesis, by reduced concentrations of non-Phe large neutral amino acids, is another cited pathological mechanism. The PKU amino acid transport model suggests Phe management should be more efficacious than is realized, as even early identified, continuously treated patients that retain therapy compliance into adulthood, demonstrate neurologic disease elements. Reduced cerebral metabolism was an early-recognized element of PKU pathology. Legacy data (late 1960's to mid-1970s) determined the Phe catabolite phenylpyruvate inhibits mitochondrial pyruvate transport. Respirometry of Pah^{enu2} cerebral mitochondria have attenuated respiratory chain complex 1 induction in response to pyruvate substrate, indicating reduced energy metabolism. Oxidative stress is intrinsic to PKU and Pah^{enu2} brain tissue presents increased reactive oxygen species. Phenylpyruvate inhibits glucose-6-phosphate dehydrogenase that generates reduced niacinamide adenine dinucleotide phosphate the obligatory cofactor of glutathione reductase. Pah^{enu2} brain tissue metabolomics identified increased oxidized glutathione and glutathione disulfide. Over-represented glutathione disulfide argues for reduced glutathione reductase activity secondary to reduced NADPH. Herein, we review evidence of energy and oxidative stress involvement in PKU pathology. Data suggests

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energy deficit and oxidative stress are features of PKU pathophysiology, providing intervention-amenable therapeutic targets to ameliorate disease elements refractory to standard of care.

Keywords

Phenylketonuria; Energy; Respiratory chain complex 1; Oxidative stress; Phenylpyruvate

1. Introduction

Prominent presentations of PAH deficient PKU are neurologic. An incompletely penetrant osteopenia phenotype manifests in a patient subset, as do emerging, incompletely penetrant cardiac and ocular phenotypes [1–4]. While expression of the PAH enzyme is restricted to liver and kidney, disease presents in tissues (brain, bone) that neither express PAH nor hydroxylate Phe. Liver and kidney tissue, sites of deficient PAH activity, display neither structural nor functional pathology. PAH deficiency leads to systemic over-representation of Phe, Phe catabolites, and Phe conjugates [5,6]. PKU pathologies are secondary to altered small molecule homeostasis, effecting susceptible cells in affected tissue [6].

The predominant mechanism attributed to PKU neurologic disease is asymmetric blood:brain barrier amino acid transport, driven by Phe over-representation. Were this the singular neurologic disease mechanism, adherence to Phe restriction should have greater effect to limit neurologic presentation. Newborn screening identifies patients enabling early intervention effectively excluding neurologic devastation. However, affected patients present residual early onset neurologic disease (loss of IQ, executive function deficit) and a plethora of late onset phenotypes (seizures, neuropsychiatric, Parkinsonism).

Over sixty years ago, PKU mitochondria involvement was recognized [7]. More recently, appreciation that oxidative stress is intrinsic to PKU has renewed interest in mitochondria involvement [8–12]. Contemporary studies in our laboratory and others have compiled evidence that mitochondrial energy deficit and oxidative stress participate in neurologic phenotypes and osteopenia. Moreover, characterization of mitochondrial involvement presents interventional opportunities. This review focuses on PKU oxidative stress and mitochondrial involvement with consideration of means whereby alternative pathway energy support and oxidative stress reduction may augment Phe restriction to ameliorate residual disease.

Traditional PKU Neuropathophysiology. The defining PKU characteristic is an elevated blood Phe concentration. Plasma Phe is central to PKU diagnostics and subsequently serves as the biochemical metric to monitor substrate-reducing management. Fig. 1 schematically relates hepatic PAH deficiency, blood Phe over-representation, amino acid interaction with the blood brain barrier LAT1 transporter, and consequences of asymmetric amino acid transport. Dopamine and serotonin paucities occur in PKU models and patients, hypothesized as a primary driver of neurologic phenotypes [13–15]. However, mental retardation is the primary consequence of untreated classical PKU while neurotransmitter deficiencies present with seizures [16]. It is unlikely neurotransmitter deficit fully explains PKU neurologic findings.

Reduced cerebral protein synthesis has been hypothesized to drive PKU neurologic disease owing to non-Phe large neutral amino acid (Leu, Ile, Val, Tyr, Trp) under-representation. Some evidence supports reduced protein synthesis, namely labeled amino acid incorporation in mouse and rat PKU models [17–18]. In patients, PET-scans with labeled amino acids suggested reduced protein synthesis [19]. Reduced protein synthesis is equated to white matter abnormalities primarily demyelination [17]. Alternatively, some evidence suggests demyelination owes to cholesterol deficiency through reduced activity of 3-hydroxy-3-methylglutryl coenzyme A reductase [20]. There are late-identified classical PKU patients that retain cognition despite years to decades of hyperphenylalaninemia and assumed protein synthesis reduction, these patients argue against a protein synthesis defect being a primary causal element [21–24].

PKU and Energy Deficit. The earliest data supporting energy deficit assessed “phenylpyruvic oligophrenia”, patients where oxygen and sugar content of cerebral arterial and venous blood were determined [25]. PKU patients displayed reduced sugar and oxygen utilization in blood having traversed the brain, interpreted to indicate diminished cerebral metabolism [25]. Early biochemical studies (1969–1976), relating PKU cerebral energy, focused on the Phe catabolite phenylpyruvate and inhibition of energy pathway enzymes. Originally, phenylpyruvate was determined to inhibit hexokinase, which creates glucose-6-phosphate being a glycolysis and pentose phosphate pathway analyte [7]. Oxidative steps of the pentose phosphate pathway produce a large portion of cerebral reduced niacinamide adenine dinucleotide phosphate (NADPH), relevant to oxidative stress as NADPH is the cofactor of glutathione reductase (see below PKU and Oxidative Stress). Moreover, the pentose phosphate pathway also produces glycolysis analytes (fructose-6-phosphate, 3-phosphoglycerate) to support energy production. The group of Dr. M.S. Patel published three manuscripts demonstrating phenylpyruvate inhibition of pyruvate carboxylase [26–28]. The reaction product of pyruvate carboxylase is the Kreb cycle analyte oxaloacetate, which upon condensation with acetyl-CoA forms citrate to drive creation of reducing equivalents (NADH, $FADH_2$) relevant to oxidative energy production. Phenylpyruvate is also described as an inhibitor of mitochondrial pyruvate transport, pyruvate kinase, and glucose-6-phosphate dehydrogenase [29,30]. Broad inhibitory activity of phenylpyruvate appears somewhat over-stated; while veracity of these data are not in question, complete relevance to PKU pathology is unclear.

The PKU rat was extensively applied in cerebral energy studies. The rat model injects newborn animals with Phe and the PAH inhibitor α-methylphenylalanine where a daily regimen maintains hyperphenylalaninemia over initial weeks of life. PKU rat studies, by Rech et al and Dimer et al, identified activity deficits in mitochondria respiratory chain complexes [31,32]. A study contradicting these found no respiratory chain deficit using hyperphenylalaninemic conditions with a human astrocyte cell line [33]. The same authors applied similar means to PKU patient blood mononuclear cells wherein respiratory complex activity was equivalent to controls [34]. These contradictory studies made assumptions limiting their utility: 1. Cell culture will replicate the in vivo brain biochemical milieu; 2. Leukocytes will share functional consequences with PKU affected tissue (e.g. brain). A recent study in succinic semialdehyde dehydrogenase deficiency determined affected tissue is the most relevant source of pathological analytes, while peripheral blood was an

unreliable source of the same analytes [35]. Our PKU comparative metabolomic study (see below) made similar observations that extended to respiratory complex 1 functional deficit [6]. It is emerging that affected tissue (e.g. PKU brain) verses a non-affected tissue (e.g. PKU blood), do not elicit equivalent response including pathway dysregulation and analyte production. Most patient studies are limited to accessible samples (blood, urine, CSF, skin biopsy); while much has been discerned from accessible sample sources, their utility to reflect outcomes realized in affected tissue may be limited.

Additional PKU rat studies sought to replete cerebral energetics. The driving force behind these studies is the group of Dr. C.M. Wannmacher at the Instituto de Ciências Básicas da Saúde, Porto Alegre, Brazil. Among their initial observations was hyperphenylalaninemia-induced, down-regulation of cerebral creatine kinase activity [36]. The role of creatine in cerebral energy homeostasis is established [37]. Studies in the PKU rat assessed concurrent application of creatine and pyruvate where follow-up included behavior characteristics, dendritic spine formation, and phosphotransferase network functionality [38–40]. They also applied innovative creatine nanoliposome delivery to demonstrate improved respiratory complex activities [41]. These studies characterize cerebral energy deficit, but more importantly, define the PKU cerebral energy deficit as treatable.

Our recent study in the Pah^{enu2} mouse further characterize energy dysregulation and oxidative stress [6]. We applied metabolomics to Pah^{eun2} blood plasma, liver tissue, and brain tissue. While liver is the principal site of PAH activity, abnormal elements of the liver analyte profile was limited to over-representation of Phe, Phe catabolites (phenylpyruvate, phenyllactate, phenylacetate, 2-hydroxyphenylacetate), and Phe-conjugates (*N*-acetylphenylalanine, *N*-formylphenylalanine, γ-glutamylphenyalanine, 1-carboxyethylphenylalanine). Liver showed no evidence of energy dysregulation. Abnormal blood analytes in Pah^{enu2} were similar to liver with Phe over-representation in addition to Phe catabolites, and Phe conjugates. No evidence of energy dysregulation was observed in Pah^{enu2} blood. The metabolomic profile of Pah^{enu2} brain tissue was unique providing evidence of energy pathway disruption with over-representation of glycolysis analytes and an increased NADH/NAD ratio. This pattern suggests a deficit in respiratory complex 1 with compensatory glycolysis up regulation. Respirometry with mitochondria prepared from Pah^{enu2} brain tissue and Pah^{enu2} liver tissue evidenced differential susceptibility to energy dysregulation. Respirometry with Pah^{enu2} and control liver mitochondria generated identical rates of oxygen consumption and response to substrates inducing complex 1 (pyruvate, glutamate) and complex 2 (succinate). Whereas mitochondria from Pah^{enu2} brain tissue showed oxygen consumption deficit in response to pyruvate substrate; however, response to glutamate substrate was similar to controls albeit beginning at a lower baseline. Pah^{enu2} and control brain tissue respond similarly to complex 2 induction with succinate substrate. Attenuated complex 1 response to pyruvate substrate is consistent with published data showing phenylpyruvate inhibition of mitochondria pyruvate transport [29]. Pah^{enu2} brain tissue analyte profile provided evidence of energy dysregulation while Pah^{enu2} liver tissue provided no evidence of energy dysregulation. These data demonstrate biochemical insult affects one or more cell populations in the Pah^{enu2} brain precipitating energy dysregulation. Cell populations in the Pah^{enu2} liver are refractory to biochemical insult relating to energy disruption. Prior studies support finding in Pah^{enu2} brain tissue [31,32]. We suggest

standard of care management inadequately remediates cerebral energy deficit, which may be contributory to residual neurologic disease.

2. PKU and oxidative stress

Oxidative stress is defined by over-representation of reactive oxygen species being in excess of intrinsic anti-oxidative buffering capacity (e.g. glutathione). Several neurologic disorders identify oxidative stress participates in disease pathology [42,43]. Low plasma selenium concentration in treated PKU patients attenuated glutathione peroxidase activity inducing oxidative stress [44–46]. PKU oxidative stress was near simultaneously described in brain tissue of the Pah^{enu2} mouse and PKU rat [8,10]. Shortly thereafter, oxidative stress was identified in PKU patient plasma [12,13]. When PKU oxidative stress was characterized, there was legacy data [26–30] but little contemporary evidence of PKU mitochondria involvement, making this an unanticipated finding. The manuscript of Sirtorri et al 2005 was highly relevant as multiple lines of evidence (thiobarbituric-acid reactive species, antioxidant reactivity, glutathione peroxidase activity) demonstrated persistent oxidative stress among managed and therapy non-complaint patients [12]. Later, Kumru et al showed Phe management moderated oxidative stress; however, even among managed patients oxidative stress persisted [47]. Systemic impact of oxidative stress was demonstrated to include DNA damage [48,49]. Assessing oxidative damage in patient brain tissue has been elusive, while surrogates for cerebral oxidative stress are identified; clear utility of these surrogates in management is not established [50–53]. Our Pah^{enu2} metabolomic investigation provide insight to tissue-specific anti-oxidative response. Pah^{enu2} brain tissue demonstrates anti-oxidative responses through glutathione and homocarnosine pathways. Oxidized glutathione and cysteine-glutathione disulfide are over-represented in Pah^{enu2} brain tissue indicating anti-oxidative response [6]. Relevant to glutathione anti-oxidative response efficacy is Pah^{enu2} cerebral representation of reduced and oxidized niacinamide adenine dinucleotide phosphate (NADP, NADPH). We determined NADP is over-represented in Pah^{enu2} brain while NADPH is under-represented. NAPDH is the obligatory cofactor of glutathione reductase that cleaves cysteine-glutathione disulfide into glutathione monomers driving continued anti-oxidative response. Rosa et al demonstrated the Phe catabolite phenylpyruvate inhibits glucose-6-phosphate dehydrogenase (G6PD) a critical source of cerebral NADPH [30]. It is possible phenylpyruvate is central to cerebral energy deficit and oxidative stress by inhibiting mitochondria pyruvate transport reducing oxidative energy production and G6PD activity denying NADPH cofactor to glutathione reductase reducing enzyme processivity. Fig. 2 diagrams how the Phe catabolite phenylpyruvate may have a central role in dysregulation of oxidative energy production and glutathione anti-oxidative response. Notable was Pah^{enu2} brain over-representation of homocarnosine and homocarnosine pathway analytes. Homocarnosine synthesis condenses histidine (His) and γ-aminobutyrate. His was over-represented in Pan^{enu2} brain with chronic over-representation evidenced by increased His conjugates (*N*-acetylhistidine, 1-carboxyethylhistidine, γ-glutamylhistidine) and the catabolite histamine. Interestingly, similar Phe conjugates (*N*-acetyl, 1-carboxyethyl, γ-glutamyl) are observed in Pah^{enu2} tissues [6]. Neuroprotective and antioxidant properties of homocarnosine are emerging making this element of PKU cerebral response a possible route to leverage therapeutically [54].

Remediating PKU oxidative stress is investigated. Carnitine has anti-oxidative properties. While carnitine is synthesized in vivo, a substantial portion is of dietary origin. As PKU management restricts carnitine rich food (meat, fish), assessing carnitine in patients and effect of carnitine supplements was determined. Well-managed patients show reduced plasma carnitine, while noncompliant or poorly compliant patients have higher carnitine concentrations, which are assumed to derive from diet [55]. While both well managed and therapy noncompliant patients showed oxidative stress, a negative correlation was demonstrated between thiobarbituric-acid reactive species and carnitine concentration [55]. Subsequently, it was shown that carnitine supplements reduce PKU patient oxidative stress [56]. Lipoic acid is an enzyme cofactor but also has anti-oxidative properties and reduces oxidative stress in the PKU rat [57]. Pyruvate and creatine were discussed above regarding energy augmentation; however, this strategy also reduced oxidative stress [40]. Other investigations have applied melatonin, vitamin E and vitamin C as PKU antioxidant regimens [58,59]. We treated Pah^{enu2} mesenchymal stem cells with resveratrol, which normalized reactive oxygen species, increased mitochondria mass, and improved osteoblast differentiation [60]. While PKU formula contains antioxidants, oxidative stress remediation has demonstrated refractivity to standard of care; normalizing the oxidative burden in PKU patients may require aggressive anti-oxidative regimens.

3. PKU osteopenia

PKU osteopenia was described in the 1960s [61,62]. Originally, PKU osteopenia was deemed secondary to diet therapy whereby calcium, phosphorous, and other bone forming material are reduced or rendered biologically unavailable; however, this is disproven as osteopenia is observed in patients that never received diet therapy and young patients after short-term therapy. The relationship between Phe and bone status remains unclear as conflicting studies indicate PHE correlates with osteopenia [63–70] while, others show no correlation [71,72]. The majority of PKU osteopenia literature is clinical and descriptive.

The first PKU osteopenia mechanistic studies assessed imbalanced bone formation and bone resorption. Osteoclastogenesis in PKU patient peripheral blood was greater in patient mixed leukocyte cultures, assayed by tartate resistant acid phosphatase staining [73,74]. In the recent decade, there has been no further published evidence substantiating these findings.

Our group hypothesized PKU osteopenia owed to a mesenchymal stem cell (MSC) developmental deficit in the osteoblast lineage. While osteopenia is not fully penetrant in patients, the Pah^{enu2} mouse is universally osteopenic. Our initial Pah^{enu2} MSC study showed an osteoblast developmental deficit, defined at cellular and molecular levels [75]. The follow-up study demonstrated Pah^{enu2} MSCs contain increased superoxide reactive oxygen species, deficient mitochondria functional metrics, and a deficit in pyruvate induction of respiratory complex 1 [76]. Similar to the brain, bone is an “affected tissue” wherein oxidative stress and energy deficit are identified. Unique to PKU MSC involvement is direct circulatory exposure mediates small molecule insult without selection as mediated by the blood:brain barrier in brain tissue. Glycolysis is the primary energy source of resting and proliferating MSCs [77]. Demonstrated in mouse and human MSCs, osteoblast differentiation requires upregulation of oxidative ATP production to

support differentiation and extracellular matrix synthesis [78]. Based on our data showing mitochondria dysfunction in Pah^{enu2} MSCs, we hypothesize energy deficit is an osteopenia contributing factor. MSCs are the first cell population identified where energy deficit and oxidative stress contribute to a PKU clinical phenotype. MSCs have preferred energy substrates. Providing preferred substrates may support MSC differentiation to improve bone density. Data published by Dr. Denise Ney's group [79] and confirmed by our group [80], showed glycomacropeptide (GMP) improves Pah^{enu2} bone density. Moreover, the Ney group determined Pah^{enu2} animals managed with GMP showed increased plasma representation of glutamate (Glu) and glutamine (Gln), being MSC preferred energy substrates [81]. We interpret these data to suggest increased Glu and Gln, secondary to GMP diet, provide energy substrates to support MSC osteoblast differentiation improving bone density. Notable is Pah^{enu2} Phe homeostasis with GMP is ~750 μM. Pah^{enu2} provided amino acid defined diet have Phe homeostasis of ~200 μM, yet bone density is indistinguishable from untreated animals [80]. Plasma Phe is the singular metric defining PKU management for >50 years; however, these data suggest energy augmentation may enable superior outcomes in the context of higher Phe homeostasis.

4. Conclusions

While PKU is the paradigm treatable genetic disease, residual neurologic phenotypes are common. Early identified, continuously treated patients present lower IQ than unaffected siblings, executive function deficit, neuropsychiatric issues, increased anxiety, and increased depression [82–85]. Attention deficit-hyperactivity disorder is common among PKU patients as ~25% of the children with early-treated PKU receive stimulant medication for attention deficit-hyperactivity disorder compared to 7% of children with diabetes and 6% of children in the general population [86]. PKU is unequivocally treatable; however, the need for means with greater efficacy is obvious.

Defined pathophysiology enables evidence-based intervention. We put forward the entirety of PKU pathophysiology is unrealized. Moreover, currently recognized consequences of asymmetric blood:brain barrier amino acid transport (Fig. 1) is unlikely to represent all relevant neuropathological mechanisms, in part explaining residual neurologic disease. While reducing Phe homeostasis will remain central to PKU intervention, characterizing pathophysiology will identify unappreciated/under-appreciated interventional routes. This review engages legacy data to that recently reported, regarding two elements of PKU pathology: 1. Oxidative stress and 2. Energy involvement. We contend intervention targeting affected tissue to support energy and reduce oxidative stress will augment Phe reduction to improve outcomes.

PKU patients and animal models present oxidative stress. Defined roles for oxidative stress in neurologic diseases (Alzheimer's, amyotrophic lateral sclerosis) give precedent to the oxidative stress role in PKU. Comparative metabolomics identified Pah^{enu2} cerebral anti-oxidative responses. Brain susceptibility to oxidative damage is recognized [87]. We suggest oxidative stress participates in both early onset and late-onset PKU neurologic phenotypes; however, mechanisms of oxidative involvement may differ. Developmental susceptibility mediates severe early-onset PKU neurologic disease, as persistent high-level

hyperphenylalaninemia that would devastate a newborn brain does not elicit similar outcomes in adolescent/adult patients that digress to therapy noncompliance. It is likely the immature brain has reduced oxidative tolerance and anti-oxidative responses may differ from the mature brain. Metabolic codependence between brain cellular components (neurons, glia, pericyte, endothelial) and developmental processes in the immature brain (e.g. proliferation, arborization, myelination, establish neural connectivity) may alter anti-oxidative response. In PKU, clear need exists to characterize both oxidative stress and oxidative stress response relative to brain development. Late-onset PKU neurologic phenotypes (e.g. neuropsychiatric, Parkinsonism) may share elements with adult onset neurologic diseases. Age alters brain energy metabolism, hypothesized as participatory in age-related neurologic decline and classical neurologic disease (e.g. amyotrophic lateral sclerosis). PKU alters cerebral biochemical homeostasis and energy utilization in addition to increasing oxidative stress. We suggest oxidative stress participates in early onset and late onset neurologic phenotypes. Aggressive regimens with anti-oxidants able to cross the blood:brain barrier may protect against oxidative damage to blunt residual neurologic disease elements.

Diseases involving energy deficit present diverse clinical phenotypes. We posit energy deficit is a driver of PKU neurologic phenotypes and osteopenia. Primary energy deficits, forms of Leigh syndrome providing an excellent example, respond poorly to treatment. However, long chain fatty acid oxidation defects respond to alternative energy repletion as triheptanoin elegantly demonstrates [88]. Respirometry in Pah^{enu2} brain tissue and in MSCs during osteoblast differentiation present a common deficit in complex 1 induction with pyruvate substrate [6,76]. Dr. A.P. Halestrap demonstrated the Phe catabolite phenylpyruvate inhibits mitochondrial pyruvate transport [29]. Fig. 2 provides a potential mechanism, supported by previously published data, whereby phenylpyruvate is a mediator of oxidative energy deficit and oxidative stress. We vision energy deficit in PKU affected tissue involves partial pyruvate transport inhibition to reduce acetyl-CoA production with secondary Kreb cycle effect. It is logical that alternative energy substrate, entering the mitochondria independent of elements that transport pyruvate, will replete energy to improve clinical phenotypes. Fig. 2 provides a generic mitochondrial “alternative energy substrate” channel to facilitate anaplerosis. Creatine alternative energy substrate studies by C.M. Wannmacher provide evidence for efficacy of energy repletion strategies [36,38–40]. Denise Ney showed GMP improved Pah^{enu2} bone density that we suggest occurs by providing MSCs their preferred Gln and Glu energy substrates [76,79,80]. We believe ongoing investigation will identify energy anaplerosis roles to improve PKU management.

It is clear PKU standard of care management counters early-onset mental retardation. Equally clear is residual neurologic disease presents in a large majority of early identified, continuously treated patients. Osteopenia is incompletely penetrant in PKU; however, it too occurs in early identified, continuously treated patients. Energy deficit and oxidative stress are under-appreciated PKU pathophysiological elements. We hypothesize energy and oxidative stress represent interventional targets whose remediation will improve neurologic outcomes and osteopenia. MSCs and cellular populations within the brain have preferred energy substrates (e.g. Gln for MSCs) and flexibility to utilize alternative substrates (e.g. lactate via astrocyte neuron exchange). Therapeutically providing energy substrate

alternatives may support neural function or bone development. Energy utilization in PKU effected tissue is poorly characterized. Further investigation is required to clarify PKU energy dysregulation and oxidative stress to identify potential interventional modalities.

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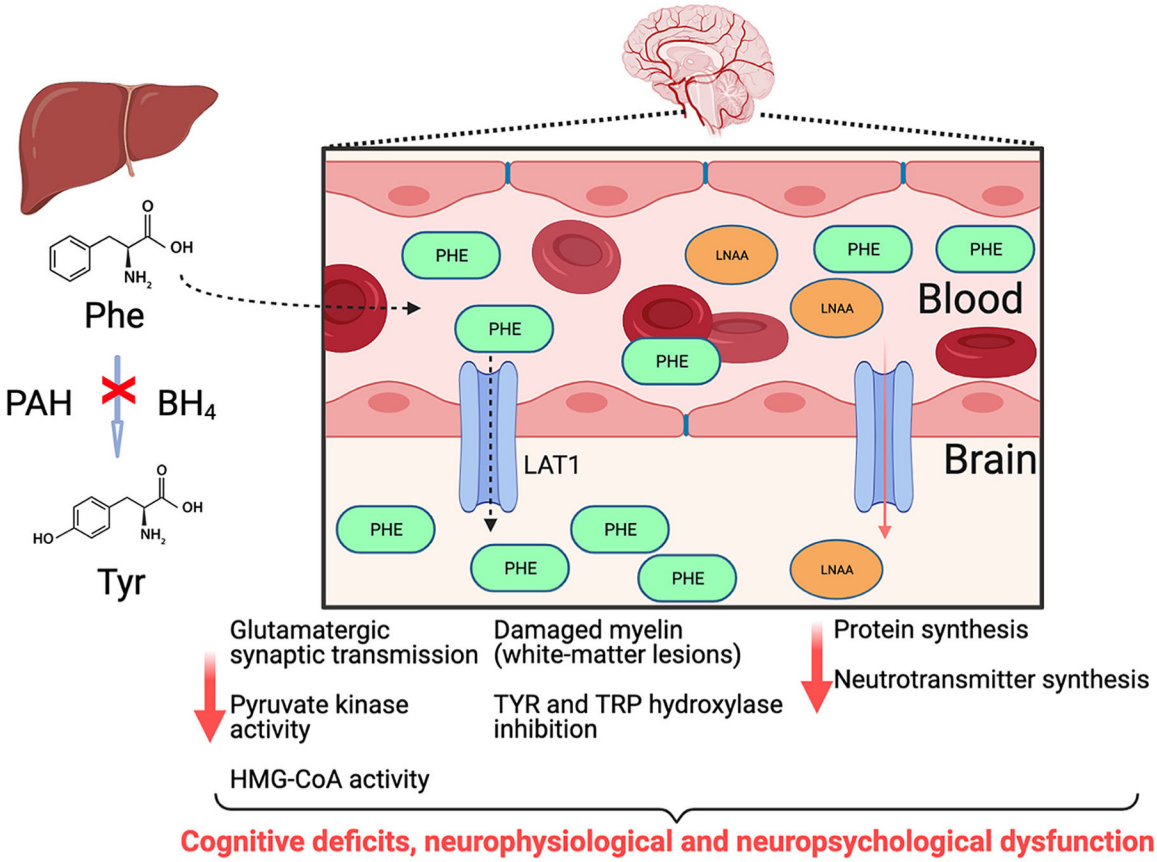

Fig. 1.

Traditional PKU pathology where hepatic PAH deficiency leads to Phe over-representation in blood. Phe over-representation leads to asymmetric amino acid transport by the large neutral amino acid transporter (LAT1, SLC7A5 gene product), with the ultimate consequence being cerebral Phe over representation and Tyr, Trp, Val, Ile, and Leu under-representation. LNAA: large neutral amino acids Tyr, Trp, Val, Ile, Leu; BH_4: tetrahydrobiopterin.

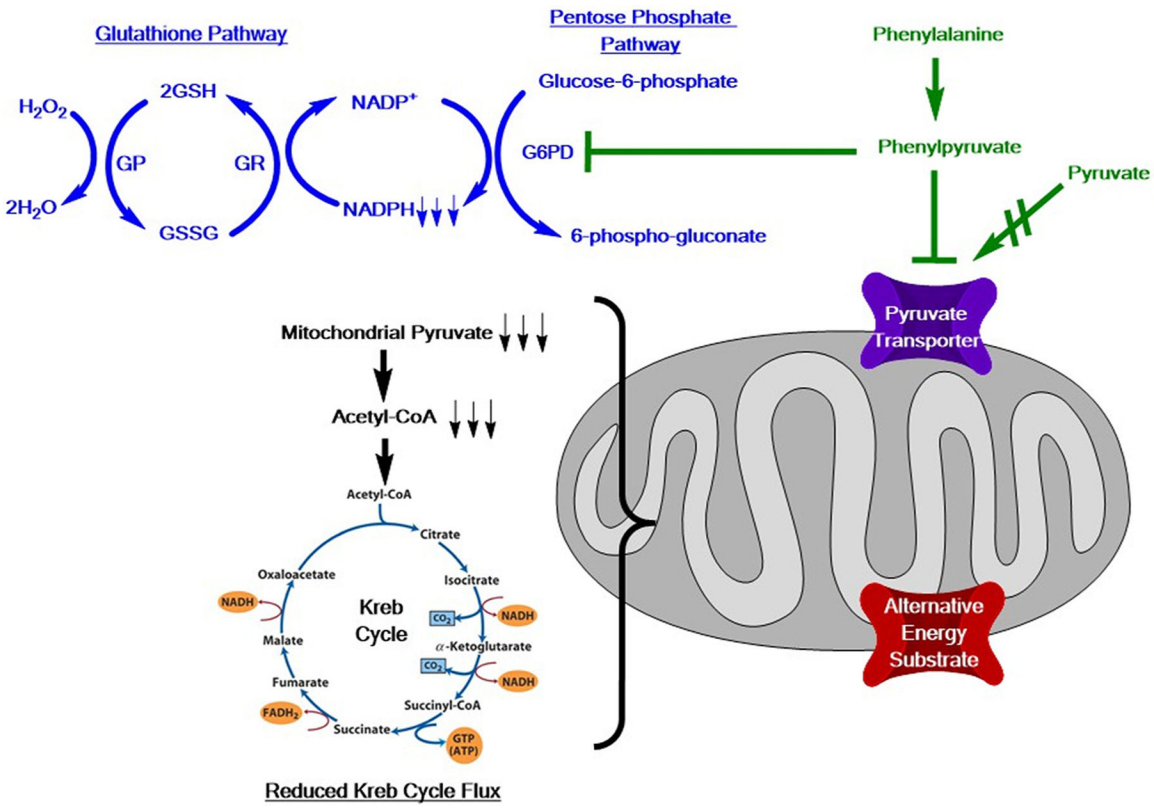

Fig. 2.

In green, Phe catabolism generates phenylpyruvate that inhibits mitochondria pyruvate transport [29] and glucose-6-phosphate dehydrogenase (G6PD) [30]. Respirometry in mitochondria from Pah^{enu2} mesenchymal stem cells [60] and brain tissue [6] show reduced oxygen consumption in response to pyruvate substrate to reduce acetyl-CoA, limiting Kreb cycle flux, and reduce mitochondria function [60]. We suggest alternative energy substrates (transporter in red), using means independent of pyruvate transporters, will replete energy production. In blue Pah^{enu2} brain tissue demonstrates reduced NADPH [6] which limits activity of glutathione reductase (GR) resulting in over-representation of glutathione disulfide (GSSG) [6]. GSH = glutathione; GP = glutathione peroxidase, NADPH = reduced niacinamide adenine dinucleotide phosphate; NADP = oxidized niacinamide adenine dinucleotide phosphate.