

## 4. Theories for Elevated Phe Levels Altering Brain Function in PKU

### Summary

- Phenylketonuria (PKU) is characterized by elevated blood levels of the amino acid phenylalanine (Phe), which is mainly obtained from eating proteins. The symptoms of PKU almost exclusively concern the brain, so how does an elevated blood Phe level affect the brain?
  - The brain is protected by a surrounding layer called the blood brain barrier (BBB), which allows some material to cross while preventing others. The brain needs certain material to cross the BBB to allow for normal functioning. One of the raw materials the brain requires are amino acids including Phe for building brain proteins and making chemical messengers called neurotransmitters.
  - The way Phe and other amino acids get across the BBB and into your brain are by using transporters. This is similar to a bus (the transporter) carrying people (the amino acids) to a destination, each seat gets taken up by an amino acid and the next stop is the brain. This analogy provides a visual description of the main theory of how elevated blood Phe can affect normal functioning of the brain.
    - Imagine that elevated blood Phe in PKU can lead to Phe taking up more seats on the bus, which may cause two things:
      - More Phe gets transported into the brain
      - Less of the other amino acids that share this bus get transported to the brain because Phe is taking up their spots!

Phenylketonuria (PKU) is characterized by elevated blood levels of the amino acid phenylalanine (Phe), which is mainly obtained from eating proteins. But the symptoms of PKU almost exclusively concern the brain. So what is going on? How does an elevated blood Phe level affect the brain? Unlocking this mystery involves understanding how Phe gets into the brain in the first place.<sup>7</sup>

First, it's important to understand that the brain is a very sensitive system. The body doesn't allow just anything floating around in the blood stream to enter it. The brain is protected by a surrounding layer called the blood brain barrier (BBB), which allows some material to cross while preventing others. Second, the brain needs certain material to cross the BBB to allow for normal functioning. One of the raw materials the brain requires are amino acids including Phe. Amino acids cross the BBB to support functions such as building brain proteins and making brain neurotransmitters. Thus, it would appear that the movement of Phe across the BBB would be important for the brain symptoms observed in PKU.

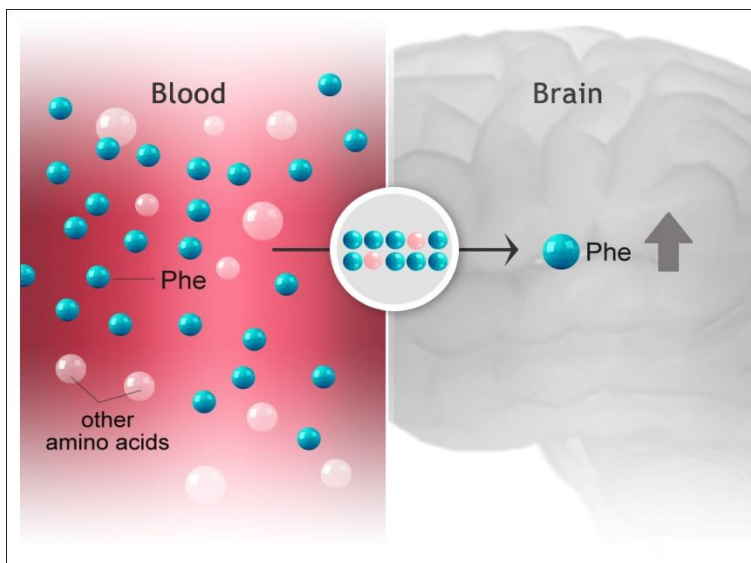
Research has shown that the transport of all amino acids into the brain involves 9 different transporters. One of these is called the large neutral amino acid transporter 1 (LAT1), which transports Phe and 8 other amino acids: valine, isoleucine, leucine, methionine, threonine, tryptophan, tyrosine, and histidine.

Think of this transporter as a bus with a limited number of seats. It picks up amino acids from the blood. When Phe levels are elevated, they compete (and win) against the other amino acids for seating. Next stop the brain! This analogy provides a visual description of the primary underlying theory of how elevated blood Phe can affect normal functioning of the brain (see Figure 5).

Simply imagine that elevated blood Phe in PKU can lead to Phe taking up more seats on the bus, which may cause two things:

- More Phe gets transported into the brain
- Less of the 8 other amino acids that share this bus get transported to the brain because Phe is taking up their spots!

**Figure 5: How Phe affects amino acid transport to the brain**

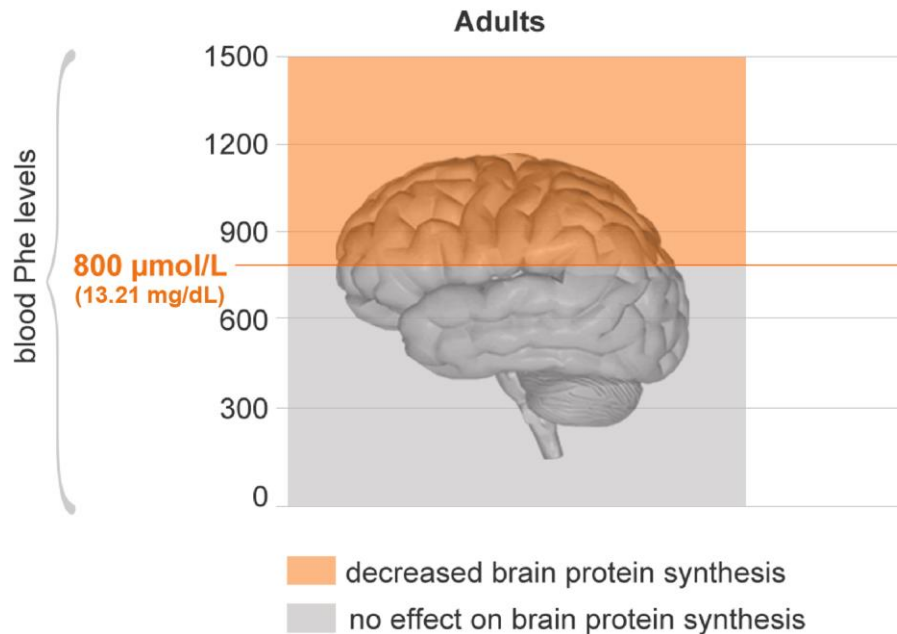


Although it is not yet technically feasible to accurately measure these amino acids in the brain, there is supporting physical and cognitive evidence for this hypothesis. There is a study that provides perhaps one of the best pieces of evidence for how elevated Phe can affect brain functioning through this transport mechanism. The study examines protein synthesis in the adult PKU brain, which relies on amino acids being transported across the BBB.<sup>33</sup> In this study, brain protein synthesis was measured in 16 PKU patients, aged 16 to 47 years ( $25 \pm 7$ ) by giving them a special identifiable form of **tyrosine** (one of the amino acids that shares the same transport mechanism as Phe), which can be measured using sophisticated brain imaging technology. At the start, individual Phe levels ranged from  $233 \mu\text{mol/L}$  ( $3.85 \text{ mg/dL}$ ) to  $1362 \mu\text{mol/L}$  ( $22.5 \text{ mg/dL}$ ) (mean:  $587 \pm 300 \mu\text{mol/L}$  [ $9.7 \text{ mg/dL} \pm 5 \text{ mg/dL}$ ]).

Study data suggests that **brain protein synthesis** in adults with PKU is abnormally low when blood Phe levels are elevated above a certain range. Protection from this potential abnormality in adulthood appears to occur at blood Phe concentrations  $<600\text{-}800 \mu\text{mol/L}$  ( $9.9\text{-}13.2 \text{ mg/dL}$ ) (see Figure 6). Thus it appears that elevated Phe can slow the transport

of tyrosine, and presumably other amino acids that share the same mechanism (i.e., transporter), into the brain. Those that had higher blood Phe levels showed poorer brain protein synthesis.

**Figure 6: Increased Phe levels decrease brain protein synthesis**



Knowing the potential mechanism of how elevated blood Phe affects the brain through altering the transport of a certain group of amino acids across the BBB helps in the understanding of how/why certain PKU therapies work:

- Restricted Phe diet and/or **PAH cofactor** (Kuvan;  $\text{BH}_4$ ) therapies: Reduces blood Phe levels resulting in Phe taking up less seats on the bus, which allows room for the other amino acids to be transported across the BBB.
- Large neutral amino acid (LNAA) therapy (e.g., vPreKUnil, NeoPhe, PheBLOC): Decreases the amount of Phe entering the brain, and increases the amount of the other amino acids which share the same transporter across the BBB. Higher levels of these other amino acids in the blood stream compete for “seats on the bus” with elevated blood Phe.