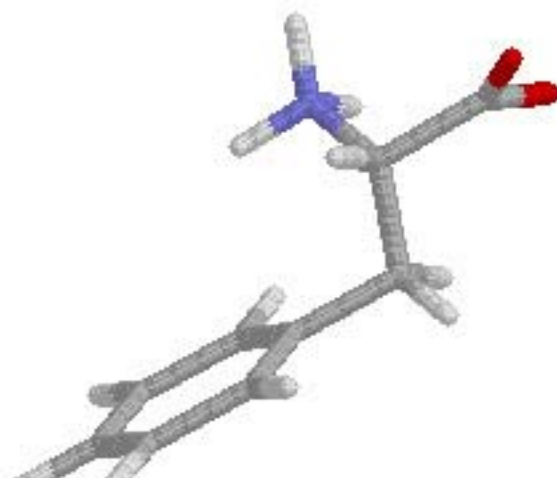


Phenylketonuria



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1)PKU Overview:

Classic Phenylketonuria (PKU) is an autosomal recessive disorder that occurs in about 1 out of every 14,000 people. It is more common in whites and Native Americans than in blacks, Hispanics, and Asians. This genetic disorder is characterized by large amounts of the amino acid phenylalanine in the blood and body tissues due to an absence, low level or deficiency of the enzyme phenylalanine hydroxylase (PAH). Under normal conditions PAH is necessary to convert the essential amino acid phenylalanine to tyrosine. Without PAH, phenylalanine cannot be broken down, and thus accumulates at toxic levels in the blood and tissues. The normal blood phenylalanine level is about 1 mg/dl. Patients with PKU typically range from 6 to 80 mg/dl, usually exceeding 30 mg/dl. Additionally, the coenzyme tetrahydrobiopterin (THB) enhances and enables the function of PAH. Defects in THB also cause hyperphenylalaninemia, which is a more rare genetic disease known as non-classical PKU.

In the United States all newborns are tested for PKU via a blood sample. However, untreated infants will generally have early symptoms within three to six months, such as vomiting, irritability, dry skin or rashes, convulsions, restless, and a hallmark feature is the musty odor to the urine. The most potentially devastating effects of PKU are neurological in nature. Children not treated for PKU will typically begin to show subtle signs of central nervous system problems like increased muscle tone and more active tendon reflexes. As the disease progresses, children are at risk for mental retardation and seizures. If treated soon after birth, these effects can be prevented.

Living with the PKU is not impossible, but it does require a restricted and intense diet extremely low phenylalanine and an adequate intake of tyrosine. The maintenance of this diet is critically important for childhood and adolescence, because increased blood levels of phenylalanine can lead to a decrease in IQ, to learning disabilities, and to behavioral disturbances in most children with PKU. Adults have some flexibility in the level of phenylalanine that can be consumed in their diet; however, it is optimal to maintain low levels. Special considerations must be taken when women with PKU becomes pregnant. While it is not critical for the woman to maintain low levels of phenylalanine for herself, it can be devastating to the fetus if it is not controlled during pregnancy. Regardless of whether the fetus has PKU or not, it can be harmed by the mother's high levels of blood phenylalanine during development. If the mother's phenylalanine is not controlled, upon birth the baby will have mental retardation and potentially microcephaly. Many also will have heart defects, low birthweight and other characteristic facial features (prominent cheeks and upper jaw bones with widely spaced teeth).

2)PKU research:

New treatments for PKU are now coming to fruition with better outcomes for patients later in life. Included in this group of treatments are methods utilizing large neutral amino acids (LNAAs). Phenylalanine is a member of this class of amino acids along with tyrosine, tryptophan, leucine, isoleucine, histidine, methionine and threonine. One of the most devastating problems caused by hyperphenylalaninemia is the neural degeneration that occurs with this disease. This is in part due to the phenylalanine

crossing the blood brain barrier through the same carriers as the other LNAA's thereby competitively inhibiting their transport. Particularly important are tyrosine and tryptophan which are substrates for the synthesis of many neurotransmitters (Dopamine, noradrenaline, serotonin, etc). Administration of high levels of LNAA's can therefore be used to bring the kinetics back to normal and lower the amount of phenylalanine entering the brain even under conditions of hyperphenylalanemia. PreKUnil is a new pill containing large doses of LNAA's (excluding Phe). This treatment is ideal for patient populations with poor diet compliance including adolescents and patients diagnosed later in life. It is contraindicated for maternal PKU and young children.

Moats RA, Moseley KD, Koch R, Nelson M Jr. Brain phenylalanine concentrations in phenylketonuria: research and treatment of adults. Pediatrics. 2003 Dec;112(6 Pt 2):1575-9.

Additionally, gene therapy is currently being explored to transfer a working copy of phenylalanine hydroxylase into an organism, although this treatment is still in the preclinical stage (not ready for human testing). A transgenic mouse with mutant knockout phenylalanine hydroxylase is commercially available with which to test treatments for classical PKU. This gene can then be transfected back into the organism through either viral or non-viral vectors. Non viral therapies may utilize naked DNA packaged within a cationic liposome or DNA covalently attached to asialoglycoprotein, which is bound and internalized by a cell surface receptor. There are many more options for efficient viral transfection. The use of a recombinant adenoviral vector has been explored, however clinical trials have shown that certain individuals may mount an immune response to this vector. Therefore the immunosuppressant, FK506, is frequently given concomitantly. Retroviral and Adeno Associated viral therapies are also being explored, and presently AAV has the highest transfection efficiency. Such therapies are investigating potential expression within liver, bone marrow, skin, and skeletal muscle.

Ding Z, Harding CO, Thony B. State-of-the-art 2003 on PKU gene therapy. Mol Genet Metab. 2004 Jan;81(1):3-8.

Enzymatic replacement therapies are also being evaluated. Current strategies have focused on two enzymes; Phenylalanine hydroxylase (PAH) and plant phenylalanine ammonia lyase (PAL). Both enzymes are introduced orally and degrade Phe within the small intestine (Parenterally introduced enzymes are highly immunogenic and have a very short half-life). Therefore, these enzymes must be packaged in such a way that they survive the acidic environment of the stomach. PAL has many advantages over mammalian PAH: 1) it requires no cofactors. 2) The product of this reaction, trans-cinnamic acid is converted in the liver to benzoic acid, which is then excreted via the urine mainly as hippurate. 3) It has a wider temperature stability range. Both enzymes have proven efficacious, however the main problem limiting this therapy as a widely used treatment is enzyme stability. Once in the intestine, the enzymes are cleaved by digestive enzymes and destroyed. There are several methods to overcome that attempt to overcome this including Pegylation modification and immobilizing PAH within artificial cells, however with moderate levels of success. This strategy may prove to be a mainstay of treatment in the future, however the hurdles of enzyme stability and immunogenicity

need to be overcome. Perhaps one day patients with PKU could eat a normal diet simply by ingesting a PAL containing pill with the steak.

Kim W, Erlandsen H, Surendran S, Stevens RC, Gamez A, Michols-Matalon K, Tying SK, Matalon R. Trends in enzyme therapy for phenylketonuria. Mol Ther. 2004 Aug;10(2):220-4.

3) Clinical Vignettes:

Diagnosis of PKU in Toddlers

A 3-year-old child presents with delayed cognitive development, eczema, seizure 2 days ago, and diminished pigmentation. Since weaning at 1, the child has been fed a strict vegan diet followed by the family. Plasma amino acids revealed elevated Phe levels (15mg%; normal: 0.5-1.8mg%) and normal levels for Tyrosine and other amino acids. What do the child's symptoms/lab values indicate?

The physical symptoms of delayed cognitive development, eczema, diminished pigmentation, seizures, and Phe levels greater than 2% are all indicators of PKU. Elevated levels of plasma Phe disrupt cellular processes in the brain, such as myelination and protein synthesis, which contribute to the child's delayed cognitive development. A vegan diet avoids some protein sources (meat and dairy), but not others (ie, legumes, nuts, soy products).

Adapted from:

Helen McCune, Registered Dietician, Shands Hospital

Maternal PKU

A 23-year-old woman gives birth to a child with microcephaly, intrauterine growth retardation, dysmorphism, and congenital heart disease. The mother has PKU. What might be causing the baby's phenotype?

Women with PKU who are not on a low Phe diet when becoming pregnant and during pregnancy have babies affected with maternal PKU syndrome. High blood phenylalanine levels in the mother cause symptoms seen in this baby as well as mental retardation. Shortly after birth, the baby can be tested for elevated blood Phe using the Guthrie test, a bacterial assay for phenylalanine. Infants may initially have normal levels of Phe because increased blood Phe is cleared by the placenta; therefore, they are usually tested for PKU after 24 hours of breastfeeding.

Adapted from:

Biochemistry, 2nd Edition.

Pamela C. Champe

Richard A. Harvey

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Philadelphia.

4) Questions and answers:

1. Which of the following about PKU is false?
 - A. One can cure PKU with a liver transplant.
 - B. If challenged with phenylalanine, the blood phenylalanine levels would be substantially higher in a heterozygote than in a normal homozygote.
 - C. The goal of PKU treatment is to administer level of phenylalanine much lower than normal.
 - D. Tyrosine is a nonessential amino acid for people with PKU.

2. Generally, during an illness, the amount of administered phenylalanine for a PKU patient should be:
 - A. Increased
 - B. Decreased
 - C. Unchanged

3. In classic PKU:
 - A. the body can't use up the amino acid phenylalanine.
 - B. The enzyme phenylalanine hydroxylase (PAH) is deficient.
 - C. The cofactor tetrahydrobiopterin is deficient.
 - D. A and B are true
 - E. All of the above are true

4. Which of the following statements about PKU is false?
 - A. If the probability of carriers for PKU is 1 of 50, and if only one parent is known to be a carrier, the probability of child having PKU would be $\frac{1}{4}$.
 - B. People that carry only one PKU gene show no sign of the disease.
 - C. Both parents must be heterozygous to produce a child with PKU
 - D. PKU is caused by mutations in both alleles of the gene for phenylalanine hydroxylase.

5. The goal of PKU treatment is:
 - A. to maintain the blood levels of phenylalanine between 30-60 mg/dl.
 - B. To administer an amount of phenylalanine that is much lower than normal.
 - C. To administer an amount of phenylalanine that is within the range of what would normally be ingested.
 - D. Both A and B are true.
 - E. All of the above are true.

6. Currently in the US, the initial presenting symptoms of PKU are:
 - A. microcephaly
 - B. heart defects
 - C. mental retardation

D. none of the above

7. At birth, an infant with PKU can be recognized by:

- A. microcephaly
- B. odor to the urine
- C. both
- D. neither

8. In one potential therapy for PKU, which amino acid(s) should be administered at high doses?

- A. tyrosine
- B. tryptophan
- C. leucine
- D. histidine
- E. all of the above

5) Answers:

1. **The correct answer is D.** People with PKU cannot synthesize tryptophan because they are missing PAH, the necessary enzyme. A is true because PAH is synthesized in the liver, so if you replace the liver you will have the enzyme. B is true because heterozygotes have only one good copy of PAH so it will take them longer to convert the phenylalanine—that is how carriers are identified.

2. **The correct answer is B.** Fever and illness can cause normal body proteins to break down, the liberation of the body's own amino acids, and thus, a rise of the blood phenylalanine level. This has to be compensated for by decreasing phenylalanine intake and administration.

3. **The correct answer is D.** deficiency in tetrahydrobiopterin is more general than classic PKU. It refers to hyperphenylalaninemia, high blood phenylalanine levels, along with a deficiency in other reactions that involve tetrahydrobiopterin. Classic PKU refers specifically to a deficiency in PAH.

4. **The correct answer is A.** PKU is autosomal recessive, so if both parents are carriers the probability of child having the disease is $\frac{1}{4}$, but if it is not known if one parent is a carrier, the probability is much less. Since PKU is recessive, but copies of PAH have to be missing, so D is true.

5. **The correct answer is B.** Since people with PKU are missing the necessary enzyme to break down phenylalanine, administration has to be much lower to preserve the same blood levels of 1mg/dl. Thus, ingestion has to be much lower than normal.

6. **The correct answer is D.** Infants with PKU appear normal until they are a few months old. In the US all babies are screened for PKU before they show any symptoms.

Thus there are no initial presenting symptoms (There may be some subtle symptoms, but are not abundantly clear until about three months).

7. **The correct answer is D.** A is false because microcephaly is a symptom of maternal PKU, not that of the baby. B is false because it takes a few months for any initial symptoms to appear in a baby with PKU.

8. **The correct answer is E.** Mental retardation in PKU is due to the fact that phenylalanine uses the same transporter to get to the brain as some other amino acids, and if phenylalanine levels are high, the transport of other amino acids will be competitively inhibited. One treatment is to increase the levels of certain other amino acids so they can compete for the transporter. These other amino acids are ones that have the same properties as phenylalanine—those belonging to the class of large neutral amino acids (LNAA). All listed amino acids belong to this category.