

## Phenylketonuria (PKU)

- classic “inborn error of metabolism”
- autosomal recessive disease characterized by mutations in the liver enzyme, **phenylalanine hydroxylase**, encoded by the **PAH** gene

PAH converts phenylalanine to tyrosine  
(reaction requires  $O_2$  and co-factor **BH<sub>4</sub>**)

- HPA or non-PKU hyperphenylalaninemia are related disorders of phenylalanine hydroxylation involving several enzymes necessary for the synthesis and recycling of co-factor for PAH, tetrahydrobiopterin (**BH<sub>4</sub>**)
- incidence: 1 in 10,000

## History of PKU

**1934:** Asbjorn Folling described an inherited metabolic disorder characterized by severe intellectual impairment, motor problems and skin abnormalities - affected individuals identified by abnormal excretion of phenylpyruvic acid (high frequency of consanguinity in parents of PKU patients; Mendelian disease)

**1950s:** PKU patients shown to have deficient activity of PAH

**1960s:** treatment of PKU with low phenylalanine diet shown to be effective

**1980s:** mapping and cloning of PAH gene

**1990s:** PKU more than a simple Mendelian trait; also behaves as complex, multifactorial disorder

**2000s:** Non-dietary treatments for PKU developed

PKU has a multifactorial cause:

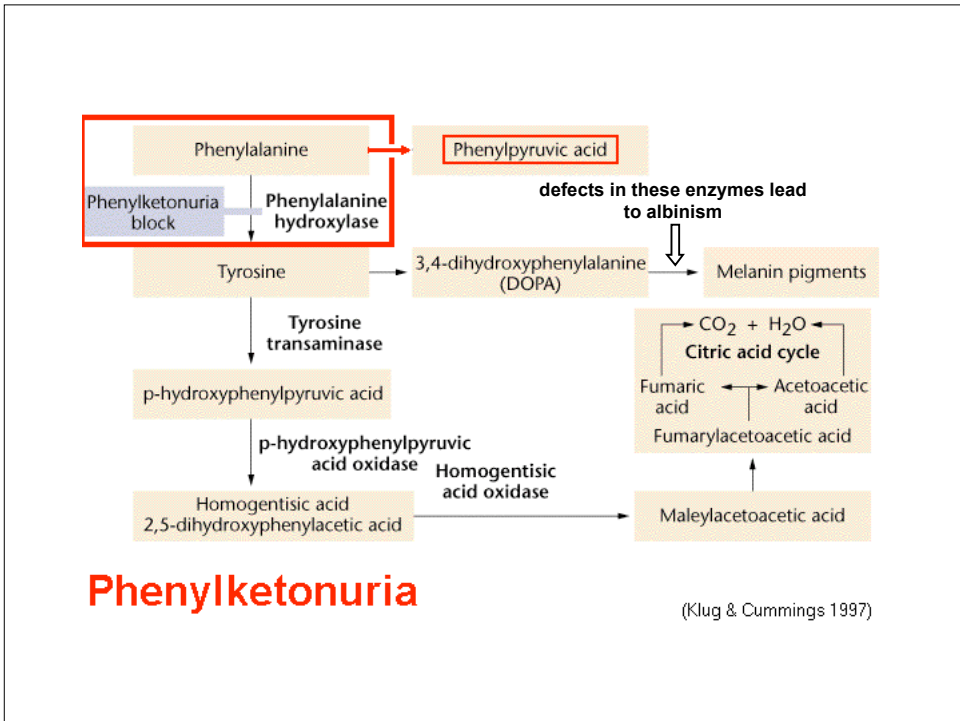
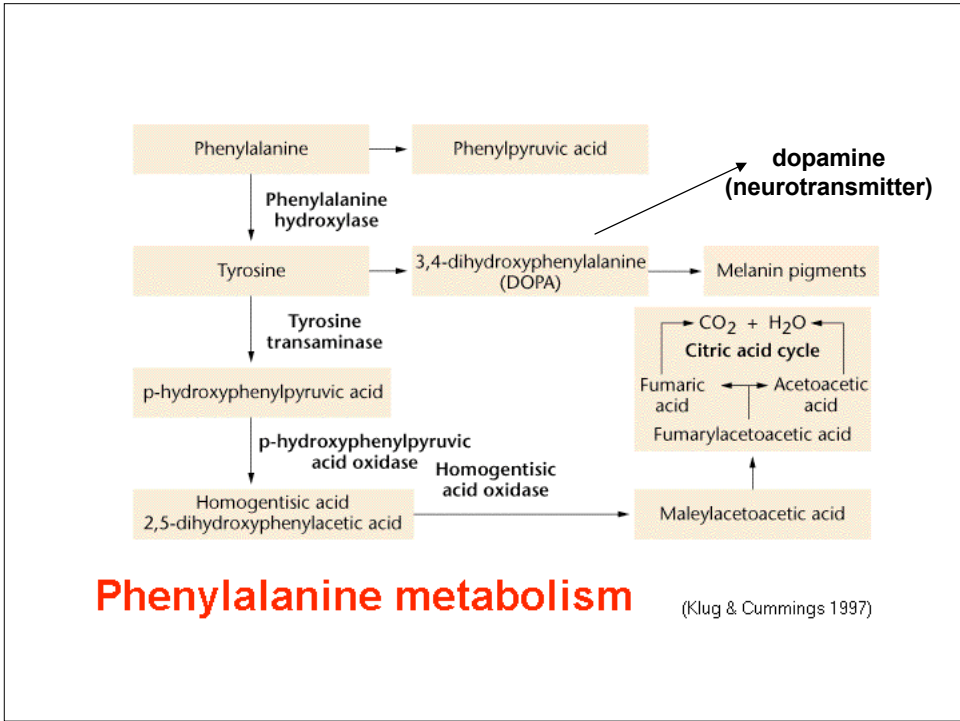
mutation in PAH gene (genetic)

exposure to dietary phenylalanine (environmental)

## Clinical features of PKU

enzyme deficiency is a primarily hepatic phenotype but major clinical presentation is abnormal brain development and function

- **reduced higher-brain abilities (executive functions)**
- **neuropsychological dysfunction (imbalance of neurotransmitters)**
- **emotional disturbance and behavioral problems (clinical depression)**
- severe mental retardation will result in untreated cases (estimated that 1% of patients in mental institutions have PKU)



### Subtypes of PKU, phenylalanine levels & clinical outlook

| subtype                    | fold increase blood [Phe]<br>(over normal) | clinical picture<br>(brain dysfunction) | treatment<br>required? |
|----------------------------|--|---|------------------------|
| classic PKU<br>(untreated) | >20  | severe mental<br>retardation            | yes                    |
| mild PKU<br>(untreated)    | 10-15                                      | cognitive loss                          | yes                    |
| non-PKU<br>mild HPA        | 2-8  | normal                                  | maybe                  |

### Newborn screening for PKU

- done with a simple blood test, screening is standard in many developed countries
- resource for sampling of mutant PAH genes
- prenatal diagnosis is possible
- classification of severe and less severe forms as well as non-PKU HPA requires Phe and BH<sub>4</sub> measurements in several body fluids

## Maternal PKU

- pregnant mothers with untreated PKU can give birth to children with severe defects

- *congenital malformations*
- *microcephaly*
- *severe mental retardation*

- careful treatment with diet is compatible with normal outcome for fetus

## Pathogenic PAH alleles

- null alleles or gene deletions (no activity)
- $V_{max}$  alleles (reduced activity)
- kinetic alleles (altered affinity for substrate or cofactor)
- unstable alleles (increased turnover and loss of PAH protein)

**majority of mutations**

Effects of disease-causing PAH mutations on a patient can be measured at three levels:

- proximal (enzymatic) : in vitro assay
- intermediate (metabolic) : plasma phenylalanine levels
- distal (cognitive function) : IQ tests

genotype-phenotype correlations show good correlations at the proximal levels

at intermediate and distal levels, phenotypes behave as complex traits suggesting the presence of “modifiers”

## Pathophysiology of PKU

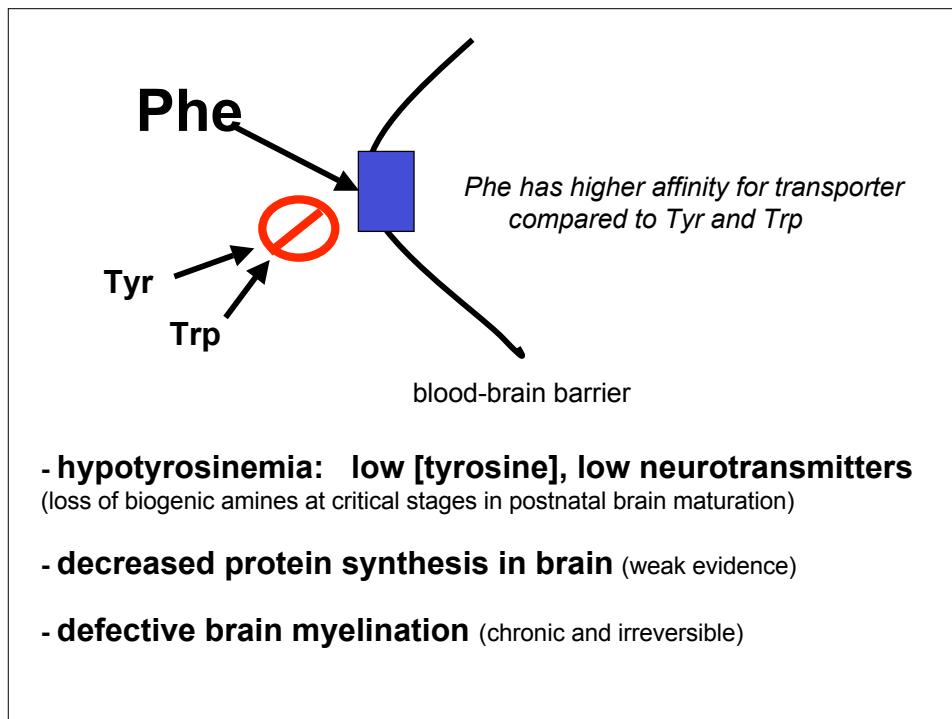
- metabolites of PKU (i.e. phenylpyruvate) not present in high enough concentrations to be toxic

- is phenylalanine the neurotoxic agent?

1) brain protein synthesis

2) transport processes and neurotransmitter biosynthesis

(tyrosine (Tyr) and tryptophan (Trp) are transported across blood-brain barrier for synthesis of the neurotransmitters, dopamine and serotonin, respectively)



### Potential problems with the low tyrosine theory...

- postnatal tyrosine supplementation without reduction of phenylalanine intake does not prevent mental retardation in PKU
- no consistent or pathological reduction in plasma tyrosine content in untreated PKU patients
- tyrosine supplements during treatment of PKU sufficient to increase plasma tyrosine levels do not improve neurophysiological parameters

## Treatment: dietary restrictions

adherence to a phenylalanine-free diet postnatally can prevent mental retardation and improve behavior in children with PKU

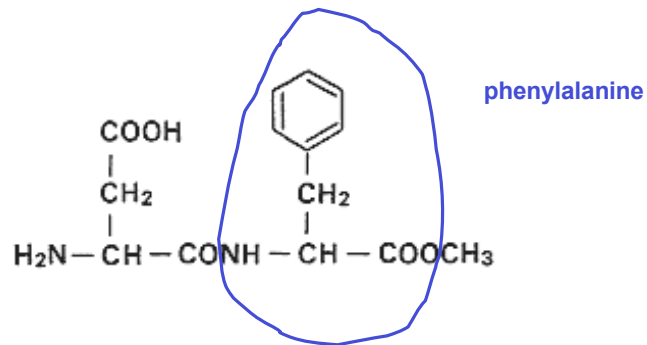


synthetic dietary supplement needed to avoid malnutrition

**Phenyl-free: Phe-free amino acid mixture, vitamins, minerals, fat** (marketed by Mead Johnson)

- offensive in odor and taste
- must be continued for life
- emotional stress in PKU families
- high cost ("patient years")

**Aspartame (Nutrasweet™)** is an amino acid sweetener, with two constituent amino acids, aspartic acid and **phenylalanine**, both commonly found in food.



synthetic diet not perfect...

- produces several biological side effects due to periodic nutrient deficiencies
- needs improvement in organoleptic properties (essential fatty acids) and nutrient composition (ratios of amino acids)

**treatment alternatives:**

- gene therapy (not yet applicable)
- enzyme replacement therapy (PAL and PEG-PAL papers)

PAL: non-mammalian enzyme; degrades Phe to ammonia and trans-cinnamic acid (harmless metabolite)

treatment of mild PKU with tetrahydrobiopterin (BH<sub>4</sub>) loading

- several recent studies suggest that BH<sub>4</sub> can be a treatment alternative to dietary restriction of phenylalanine

**Tetrahydrobiopterin as an alternative treatment for mild phenylketonuria**  
*N Engl J Med. 2002 Dec 26;347(26):2122-32*

- out of 38 with PAH deficiency, 87% showed responsiveness to BH<sub>4</sub> (i.e. had lower blood phenylalanine levels)
- no response in 7 patients with classic PKU
- long-term treatment with BH<sub>4</sub> in 5 patients increased daily phenylalanine tolerance enough to discontinue Phe-restricted diet
- mutations connected to BH<sub>4</sub> responsiveness predominantly in the catalytic domain of the protein and were not directly involved in cofactor binding

**Treatment of classical PKU with BH<sub>4</sub>**

- recent reports indicate that BH<sub>4</sub> loading was also beneficial to patients with more severe forms of PKU not just mild non-PKU HPA

38 US PKU patients were given single dose of BH<sub>4</sub> and Phe levels were monitored

58% responded at 24 h (>30% decrease in Phe levels); some who responded favorable were clinically described with classical PKU

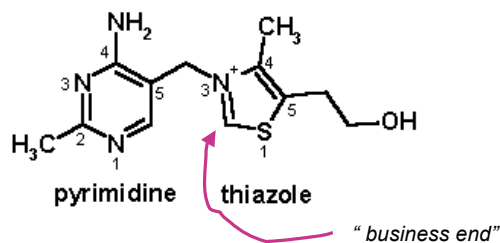
mutant PAH responds with increase in the residual enzyme activity following BH<sub>4</sub> administration

- increased stability
- chaperone effect (better folding)
- correction of mutant Km

**Kuvan™**: synthetic form of BH<sub>4</sub> that is approved in Europe for treatment of non-PKU HPA

## Diet and disease: vitamin deficiencies

### Beriberi - vitamin B-1 (thiamin) deficiency



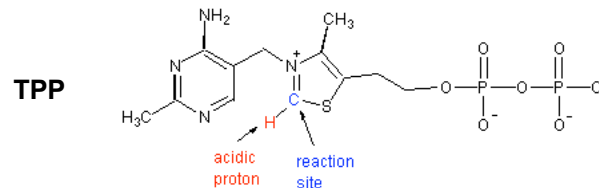
whole\* grains and lean pork# are a good dietary source

# Japanese navy and Dr. Takaki

\* Christiaan Eijkman, chickens

[http://nobelprize.org/medicine/educational/vitamin\\_b1/eijkman.html](http://nobelprize.org/medicine/educational/vitamin_b1/eijkman.html)

- thiamin (vitamin B-1) in its pyrophosphate form acts as a coenzyme in the decarboxylation and transketolation pathways (pentose phosphate pathway) of carbohydrate metabolism, and possibly in nerve conduction (essential for the synthesis of acetylcholine)



*when proton dissociates a carbanion is formed which readily undergoes nucleophilic addition to carbonyl groups*

- affects the cardiovascular, muscular, gastrointestinal, and nervous systems (weight loss, cardiac abnormalities and neuromuscular disorders such as tremors)
- pathophysiology of clinical manifestations of beriberi not known

## Rickets - vitamin D deficiency

- caused by insufficiency or inefficient action of activated vitamin D in the body during childhood
- vitamin D is fat-soluble vitamin either absorbed from intestines or produced by the skin when skin is exposed to sunlight; converted to its active form in two steps
- in its active form, vitamin D acts as a hormone to regulate calcium absorption from the intestine and to regulate levels of calcium and phosphate in the bones
- deficiency causes progressive softening and weakening of the bone structure

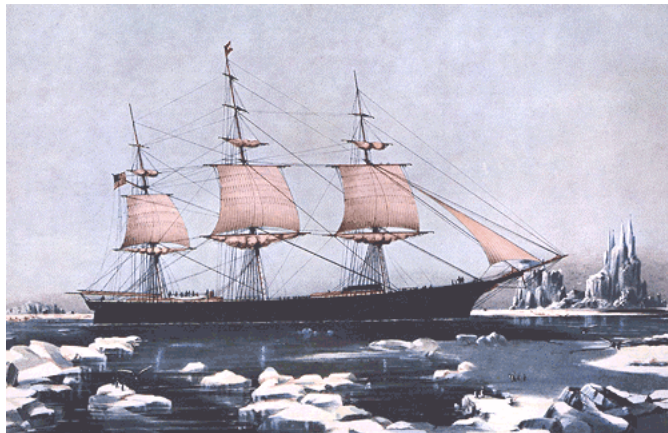
**hereditary rickets, an inherited, sex-linked disorder, is a vitamin D-resistant form of rickets caused mutations in the vitamin D receptor (VDR); vitamin D-resistance prevents kidneys from retaining phosphate**

## Scurvy: vitamin C deficiency

- sailors at sea for long periods developed several debilitating symptoms

- 1) joint pain and weakness
- 2) internal hemorrhaging and bruising
- 3) loose and bleeding teeth
- 4) mental disturbances

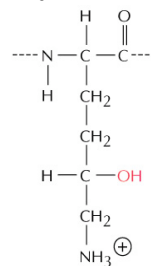
the story of a sailor “who ate grass, like a beast, and survived”



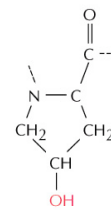
- James Lind discovered that citrus fruits could prevent scurvy and developed a method for concentrating and preserving citrus fruit juices for use at sea
- in 1795, the British Royal Navy provided a daily ration of lemon or lime juice (“limeys”)
- in 1932, vitamin C or ascorbic acid was isolated and synthesized

## Biological functions of vitamin C

- a reducing agent (or antioxidant); essential nutrient in humans, apes
- necessary to maintain the enzyme prolyl hydroxylase in its active form by keeping its iron atom in a reduced state
- enzyme converts the prolines and lysines in procollagen to their hydroxylated forms
- many other functions

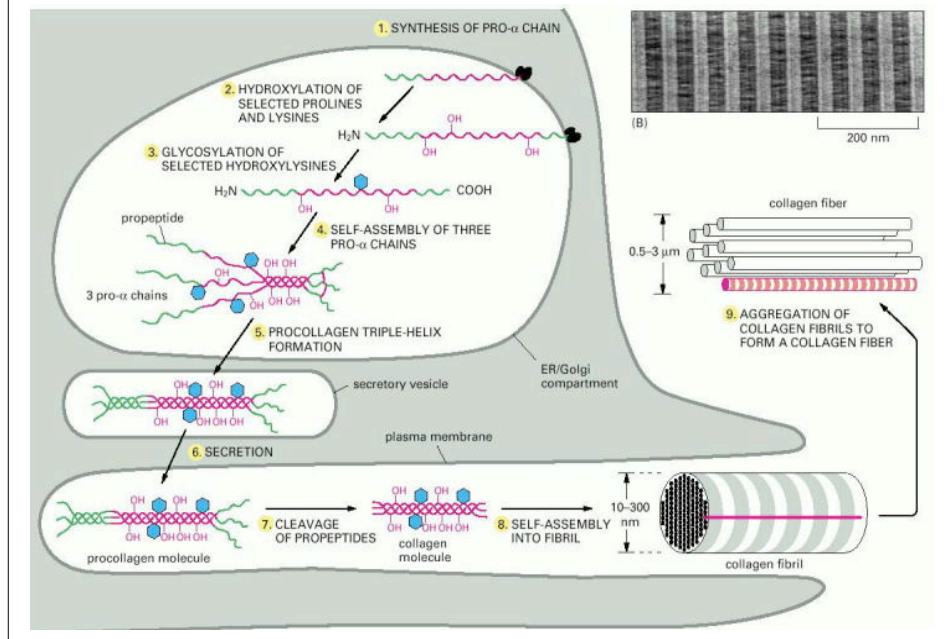


hydroxylysine  
in protein



hydroxyproline  
in protein

## Formation of collagen fibril



## Disease mechanism and pathophysiology of scurvy

- hydroxyl groups form interchain hydrogen bonds that stabilize the triple-stranded collagen helix
- underhydroxylation of proline in scurvy leads to unstable helix and immediate degradation within the cell
- collagen needed for connective tissue, bones, and dentin (major portion of teeth) as well as maintenance of blood capillary structure
- turnover of collagen in blood vessels and teeth is rapid; slow in bone

many steps in collagen processing



many genetic diseases affecting fibril formation

| <u>mutation</u>   | <u>disease</u>                        | <u>clinical feature</u>                            |
|-------------------|---------------------------------------|--|
| type I collagen   | <b><i>osteogenesis imperfecta</i></b> | weak bones   |
| type II collagen  | <b><i>chondrodysplasias</i></b>       | bone & joint deformities                           |
| type III collagen | <b><i>Ehlers-Danlos syndrome</i></b>  | fragile skin and blood vessels, hypermobile joints |

## Other examples of the relationship between diet and disease

- high fat diet and type II diabetes
- Equator and spices
- Native American Indians and succotash (corn low in lysine, beans low in methionine)
- malaria and quinine

