Hemoglobin

The big unique protein compound (come from words in Greek haima mean blood + globus mean sphere) have **64,000 daltons molecular weight**, and formed **95%** from dried RBC, but formed **34%** from RBC during movement in blood vessels. It found inside erythrocytes and responsible for carries oxygen from lung to body tissues, also transport CO_2 from tissues to the lungs. A healthy individual human has **12** to **20 grams** of hemoglobin in every **100 mL** of blood.

Hemoglobin (Hb) structure

Each human red blood cell contains approximately **270 million** hemoglobin molecules, it is a large tetrameric molecule, composed of four globular protein subunits (figure1). Each of four subunits contains; **heme** group and a **globin** chain (4 heme and 4 globin subunits).

Heme:

Is the **prosthetic** group of hemoglobin, is a complex of **protoporphyrin IX** ring and **iron**. Iron has to be in the **ferrous state (Fe^{2+})**, otherwise it will not bind to Hb, ferrous iron located in the center of the protoporphyrin ring, each heme subunit can carry **one molecule of oxygen** bound to the central ferrous iron; thus, each hemoglobin molecule can carry **four molecules** of oxygen.

Globin:

The composition of the globin polypeptide chain is responsible for the different functional and physical properties of hemoglobin. Important amino acids that formed circular polypeptide chain of globin include **Lysine, Leucine, Aspartate, Glutamate acid and Arginine.**

Four types of globin chains, **alpha α, beta β, gama γ** and **delta δ**.

Adult hemoglobin HbA composed from **2 α chains and 2 β chains**.

Alpha globin chain composed from 141 amino acids but other chains of globin contains 146 amino acids.

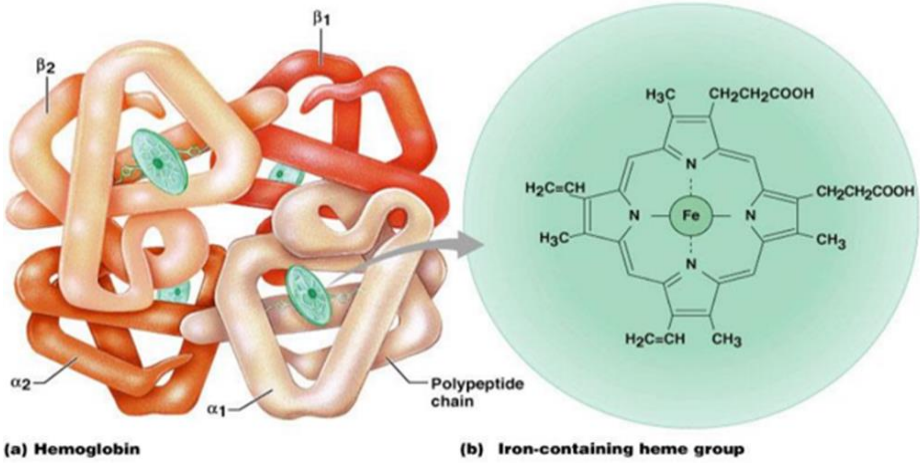


figure 1 ***Hemoglobin structure***

Hemoglobin synthesis:

About **65%** of hemoglobin synthesis occurs in the nucleated stages of RBC maturation and **35%** during the reticulocyte stage (nunucleatd). It is synthesized in a complex series of steps. The **heme** part is synthesized in a series of steps in the **mitochondria** and the **cytosol** of **immature** red blood cells, Hb synthesis process begins when binding of glycine and succinyl coenzyme A, and ends with the production of a protoporphyrin IX ring formation. The binding of the protoporphyrin to a Fe^{2+} ion forms the final heme molecule.

Glycine + succinyl Co-A ⟶ Pyrrol ring

4 Pyrrol rings ⟶ Protoporphyrin

Protoporphyrin + Fe^{+2} ⟶ Heme

While the **globin** protein parts are synthesized by **ribosomes** in the **cytosol during transcription and translation (DNA --- mRNA---- protein)**.

Production of Hb continues in the cell throughout its early development from **proerythroblast** to the **reticulocyte** in the **bone marrow**. At this stage, the nucleus is lost in mammalian red blood cells, but not in birds and many other species. Many studies have shown that the presence of heme induces globin gene transcription. Genes for the alpha chain of globin are on chromosome 16, and genes for the beta chain are on chromosome 11 of human Genomes.

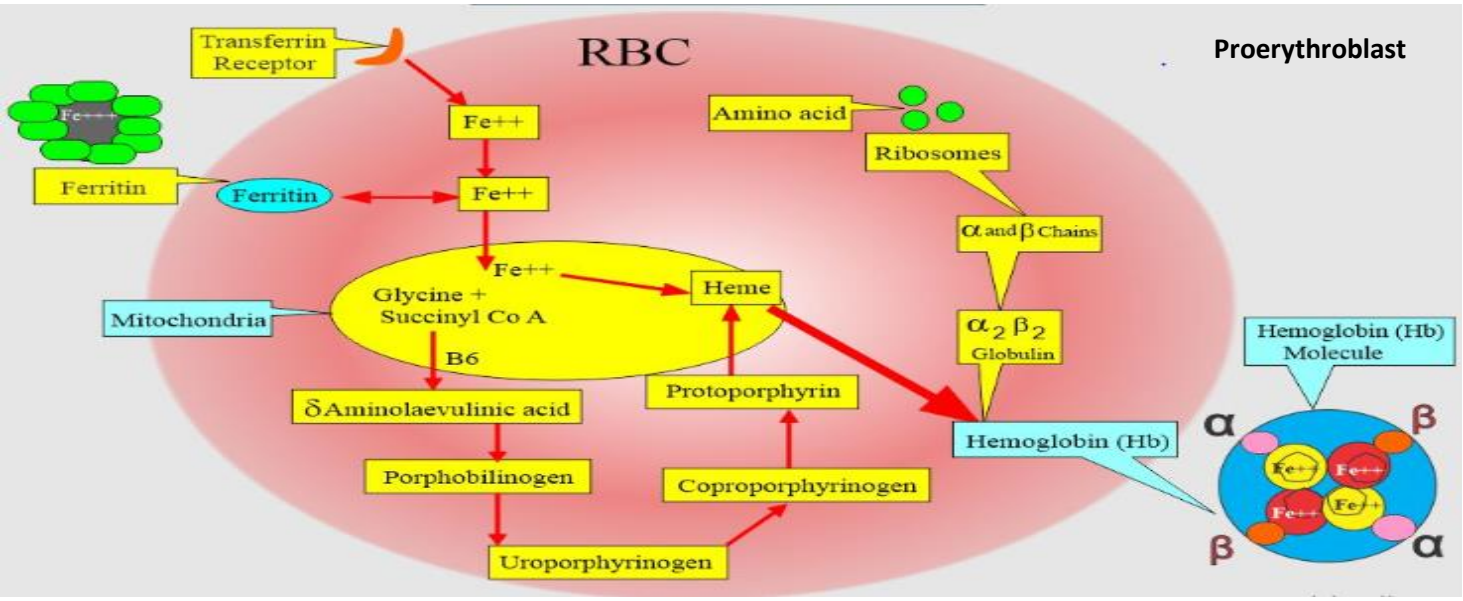


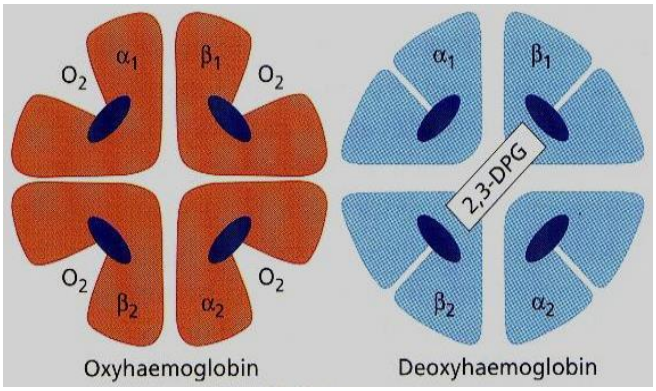
Figure 2 ***Hemoglobin synthesis***

Important factors in hemoglobin synthesis: Vitamin C, B6, B12, folic acid copper, iron, amino acids and residual of hemoglobin results from destroyed old RBC.

The four subunits of hemoglobin, each consisting of a heme group surrounded by a globin chain, are held together by salt bonds, hydrophobic contacts, and hydrogen bonds in a tetrahedral formation giving the hemoglobin molecule a nearly spherical shape.

Hemoglobin functions:

1. Hemoglobin is an oxygen carrier.



The oxygen bound hemoglobin is referred to as **oxyhemoglobin**, one gram of Hb have ability to bind with **1.34 mm** of O_2. When blood reaches an oxygen deficient tissue, oxygen is dissociated from hemoglobin and diffused into the tissue.

2. It is a carbon dioxide carrier.

Hemoglobin also transports carbon dioxide from tissues to lungs. 80% of the carbon dioxide is transported via plasma. Carbon dioxide does not compete with the oxygen binding site of hemoglobin. It binds to the protein structure other than iron binding position. The carbon dioxide bound hemoglobin is referred to as carbaminohemoglobin.

3. Maintains the shape of the red blood cells.

Red blood cells are biconcave disks which are flattened and depressed in the center. But some mutants may cause hereditary diseases like hemoglobinpathesis whch cause abnormal RBC shape.

4. Acts as a buffer saver.

Hemoglobin maintains the blood pH at 7.4. Accumulation of carbon dioxide in the blood decreases the pH from 7.4. The change of the pH can be reversed by ventilation. Due to this buffering action of hemoglobin.

5. That interacts with other ligands.

Hemoglobins also bind to other ligands such as carbon monoxide, nitrogen oxide, cyanide, sulfur monoxide, sulfide and hydrogen sulfide. Binding of carbon monoxide may sometimes

be lethal because the binding is irreversible. Hemoglobin can also transport drugs to their site of action.

6. Hemoglobin degradation accumulates physiologically active catabolites.

Hemoglobin of the dead red blood cells is cleared from the circulation by the hemoglobin transporter, **CD163**. Heme degradation, which occurs in monocytes and macrophages, is a natural source of the carbon monoxide generation. Bilirubin is the final product of heme degradation. It is secreted as bile into intestine. Bilirubin is converted into urobilinogen which is found in feces, giving the unique yellow color.

On the other hand, iron, which is removed from Hemoglobin can also be found in other cells of the body than red blood cells. Other hemoglobin carrying cells are macrophages, alveolar cells in lungs and mesangial cells in the kidney. Hemoglobin functions as a regulator of iron-metabolism and an antioxidant in these cells.

Hemoglobin types:

Hemoglobin is a heterotetramer composed of 2 α and 2 β polypeptide globin subunits, hemoglobin was one of the first proteins to be sequenced and the globin genes were among the earliest to be cloned, more than 1000 naturally occurring human hemoglobin variants with single amino acid substitutions throughout the molecule have been discovered, mainly through their clinical and/or laboratory manifestations.

Types of hemoglobin during different periods of life

Type of hemoglobin	*Globin chains*	*Period of life when predominant*
Hemoglobin Gower I	ζ 2ε2	Embryonic
Hemoglobin Gower II	α 2ε2	Embryonic
Hemoglobin Portland	ζ 2γ2	Embryonic
Hemoglobin F (Fetal hemoglobin)	α 2γ2	Fetal
Hemoglobin A (Adult hemoglobin)	α 2β2	Adult
Hemoglobin A_2	α 2δ2	Adult

A-normal types

In adults, these are normal percentages of different hemoglobin molecules:

HbA: 95% to 98% /// **HbA2**: 2% to 3%

HbF: 0.8% to 2%

In infants and children, these are normal percentage of HbF molecules:

HbF (newborn): 50% to 80% (0.5 to 0.8), decrease after 6 months: 8%, and more than 6 months become 1 - 2%.

Normal value ranges may vary slightly among different laboratories. Some labs use different measurements or may test different samples.

B-abnormal types:

After electrophoresis of hemoglobin, inherited blood disorder in which the body makes an abnormal form of hemoglobin, such as HbH (thalassemia), HbSS (Sickle cell anemia), Methemoglobin is hemoglobin with iron oxidized to the ferric state, which cannot carry oxygen and other abnormal types we will discuss later.

Factors effect on affinity between hemoglobin and O_2:

1-**PH**: the affinity of hemoglobin to oxygen is positively correlated with blood acidity (H+), the degree of hemoglobin affinity is related to the degree of acidity of the blood, a low pH of the blood means a decrease in the degree of affinity of hemoglobin to combine with oxygen.

low PH = low affinity.

2-**PCO_2** partial pressure of carbon dioxide that have inversely effect on affinity.

3-**Temperature** have inversely effect on affinity.

(these three factors, PCO_2, PH and temperature called **Bohr effect** the principle named after Christian Bohr discover it in 1904 AD).

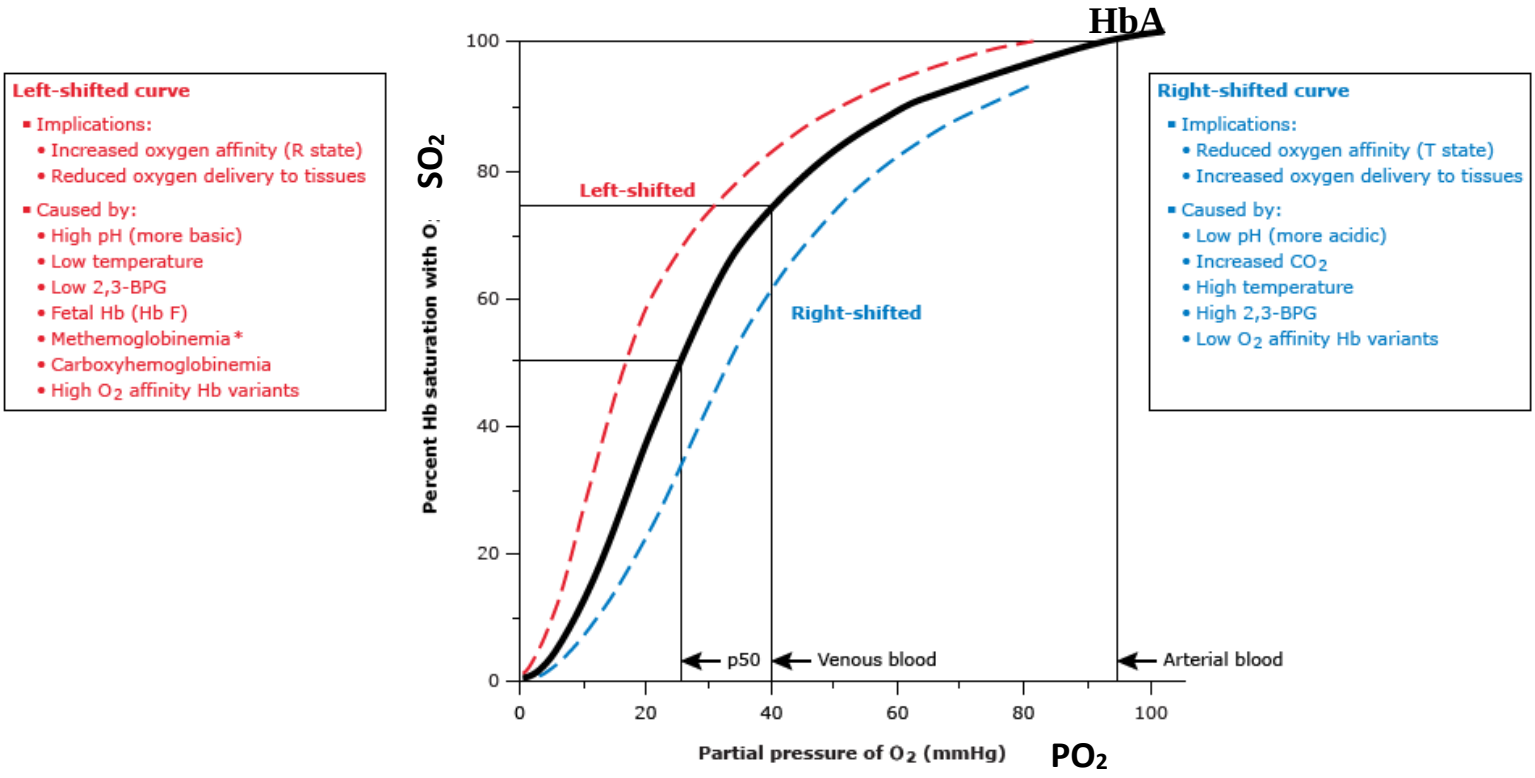
4- **2,3-BPG** concentration, also have inversely effect

5- **Carbon monoxide** CO: it is known as the silent killer since it has no colour or smell, has **210 times greater affinity for haemoglobin than oxygen**, this means that less oxygen will actually be delivered to our tissues. (make as acompetition for O_2 because that it has positive correlation).

6- **The presence of unusual hemoglobin species**, **Methaemoglobin, carboxyhaemoglobin and fetal haemoglobin** that have positive effect and **Sulfhaemoglobin** have inversely effect.

Hemoglobin Degradation, we discussed it in Erythrocyte fate

Hemoglobin oxygen dissociation curve



(Normal P50, measured at 37°C and an arterial pH of 7.40, is **27 mm/Hg)**.

When 50% of Hb saturated with O2 (SO_2) = PO_2 is 27 mm/hg

Venous blood: SO_2=75 and PO_2=40

Arterial blood: SO_2 =100 and PO_2 =100

By increasing the hydrogen ion concentration (and therefore the pH), the temperature, the carbon dioxide concentration or the amount of 2,3-BPG present in the red blood cell, we ultimately decrease the affinity of hemoglobin to oxygen, therefore these factors have negative (inversely) effect on affinity between O_2 and Hb.

Normal values of Hemoglobin:

Normal results for adults vary, but in general are:

Male: 13.8 to 17.2 g/dL

Female: 12.1 to 15.1 g/dL

Newborn: 14 to 24 g/dL

Infant: 9.5 to 13 g/dL

Hemoglobin derivatives:

When hemoglobin bind with substances other oxygen produces:

1-Meta-hemoglobin: that formed when Hb bind with KCN (potassium cyanid), Fe^{+2} converted to Fe^{+3} and heme converted to hemin

2-Caboxy-hemoglobin: formed after CO bind with Hb

3-Sulf-hemoglobin: come from bind of H2S with Hb

All these compound are toxic because incapable to transport O_2.

α- cluster	β- cluster
1. Chromosome 16 contain the following chains: a ; ζ	1. Chromosome 11 contain the following chains: ε,δ,γ,β
2. Has 141 amino acids	2. Has 146 amino acids
3. They are essential for life	3. they are not essential for life
4. Inherited 2 from each parent	4. Inherited one gene from each parent

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