Revised & Approved

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BIOCHEMISTRY TEAM
439

MED439
KING SAUD UNIVERSITY

Structure and function of haemoglobin

Editing File

Color Index

- Main Text
- Important
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- Dr.'s Notes
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Objectives

- The structure and function of hemoglobin
- The factors affecting oxygen binding to hemoglobin
- Examples of normal and abnormal hemoglobin structures

Hemoglobin (Hb)

1. A hemeprotein found only in red blood cells
(Class of different proteins which have protein part -Globin chain- and non protein part -heme group- attach)
2. Oxygen transport function
3. Contains heme as prosthetic group
(Prominently attached with globin chain).
4. There are 4 heme molecules in each hemoglobin,
Heme reversibly binds to oxygen
(It's important for binding to oxygen -Load- then release it -unload- in the tissue).

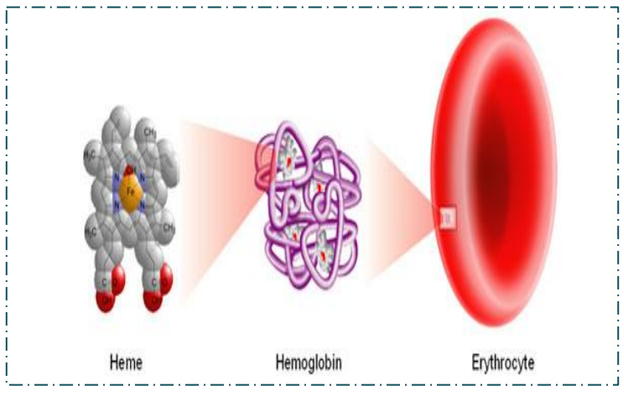
The heme group

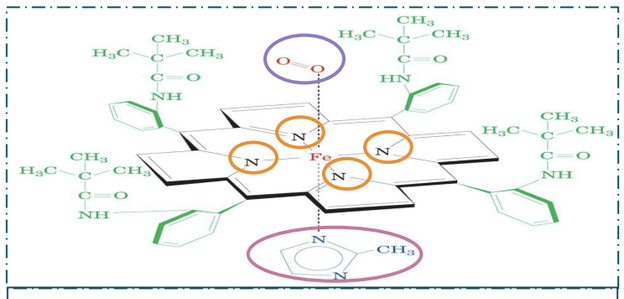
(The function of heme group depends on the protein part attached, for example in cytochrome the function is oxidation reduction but in catalase enzyme the function of heme group is breaking down the hydrogen peroxide)

It is a complex made of: **Protoporphyrin IX and Ferrous iron** (Fe^{2+})

Fe^{2+} is present in the center of the heme and binds to 6 different molecules.

Fe^{2+} binds to six molecules because it's in ferrous state:





The heme group:
Fe^{2+}-Porphyrin complex with bound O_2

- Four **nitrogen** atoms of the porphyrin ring.
- **Histidine residue of globin chain**
- **Oxygen** (1 Oxygen molecule = 2 oxygen atoms)

If the iron in the oxidized ferric state it can make only five bonds (4 with nitrogen, and one with globin chain) so there is no place for the oxygen.

Types of Hemoglobin

Normal Hemoglobin

We call them normal because they can bind to oxygen

Form Dr.Sumbul: You have to know the compositions and the fraction for all of them	Chain Composition	Fraction of Total Hemoglobin
HbA	$\alpha_2\beta_2$	90%
HbA1c	$\alpha_2\beta_{2\text{ - Glucose}}$	3% - 9%
HbF **F = Fetal** Major form in the fetus, after the birth the HbA become the major form	$\alpha_2\gamma_2$ (2 alpha and 2 gamma)	<2%
HbA2 Before birth	$A_2\delta_2$ (2 alpha and 2 delta)	2% - 5%

Abnormal Hemoglobin

They can't bind to oxygen (For example Met-Hb and Sulf-Hb), or they can't release oxygen (For example Carboxy-Hb)

The following are unable to transport oxygen due to abnormal structure

Met-Hb

Contains oxidized ferric acid Fe^{3+} (~2%) that cannot carry O2.
Normally found in the body in small amount

Carboxy-Hb

Carbon monoxide (CO) replaces O2 and binds 220X tighter than O2. (Seen in smokers) (that's why they have difficulty in breathing sometimes)
Can found in case of carbon monoxide poisoning, and we normally get it in small amount from pollution

Sulf-Hb

- Forms due to high sulfur levels in blood (Maybe caused by drugs that increasing the sulfur)
- Irreversible reaction causes cyanosis.

Structure of Hemoglobin A (HbA)

- It is the **major** Hb in adults
- Composed of 4 polypeptide chains:
  - **2 α** chains and **2 β** chains
- Contains 2 dimers of αβ subunits, held together by noncovalent interactions
- Each chain is a subunit with a heme group in the center that carries oxygen
- A hemoglobin molecule contains 4 heme groups and carries 4 molecules of O2 4 O2 molecules = 8 oxygen atoms

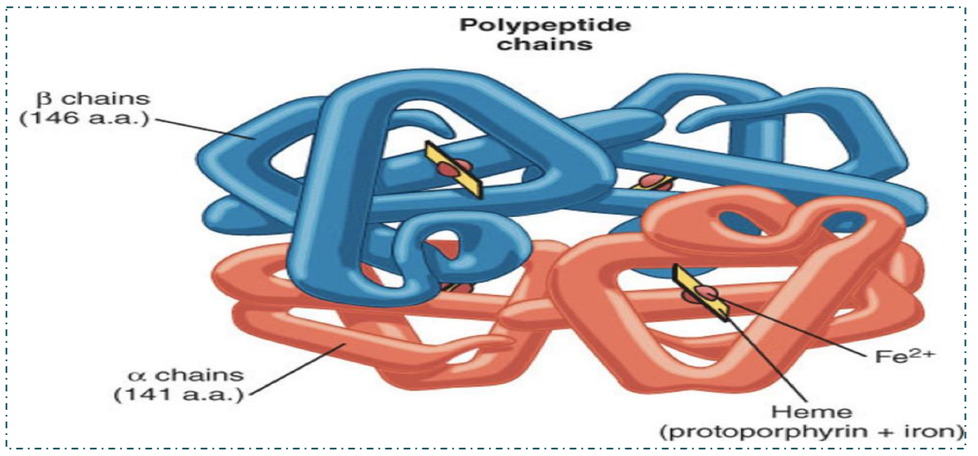
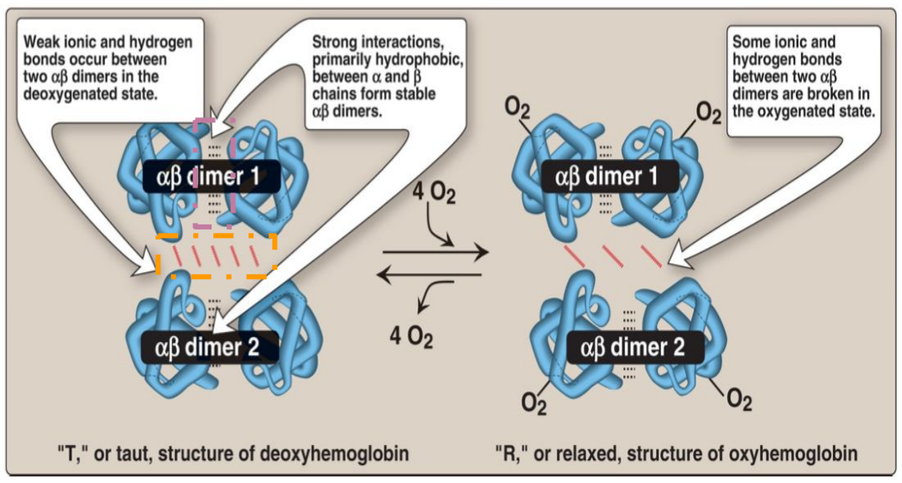


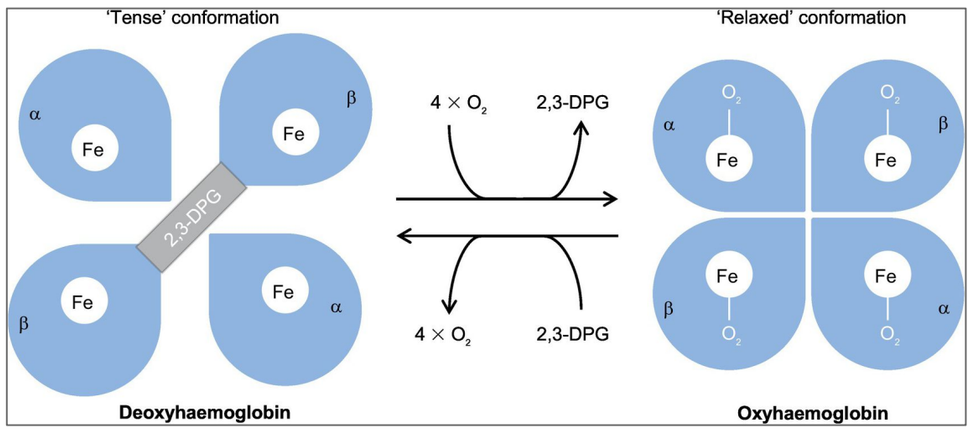
Figure Comments: 439 Respiratory Block
There are two types of bonding in the HbA structure:

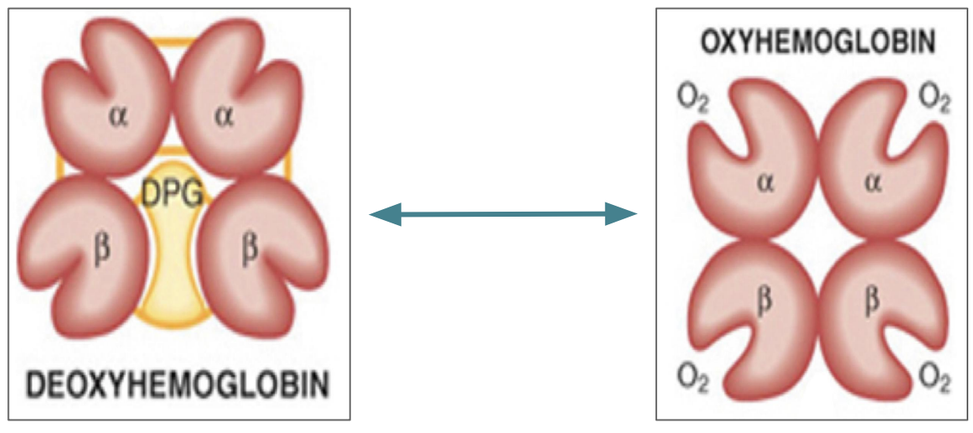
- **Intra**-dimer bonding: strong bonds between two subunits (between α and β) (hydrophobic bond)
- **Inter**-dimer bonding: weak bonds between two dimers (between αβ and αβ)



Forms of Hemoglobin

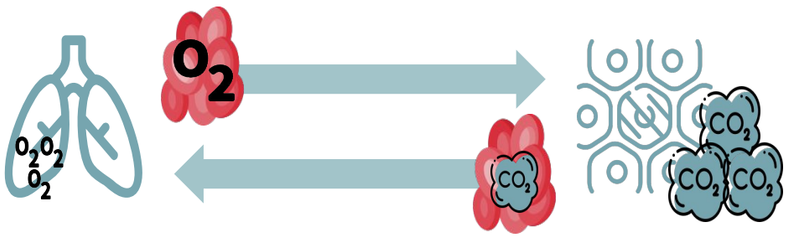
T- Form Found in tissues	**R- Form** Found in lung
Deoxy form of Hb When there is NO oxygen bound to it	Oxygenated form of Hb
“Taut” or “Tense” form	Relaxed form
The movement of dimers is constrained	Dimers have more freedom of movement
Affinity in general means how much strongly the Hb holding on the Oxygen molecule, can the Hb unload the oxygen in periphery or not?	
Low oxygen affinity form Low affinity leads to release O2	High oxygen affinity form High affinity makes the Hb hold on the O2 and can’t unload it (can’t release it)
Abundant 2,3-DPG or also known as 2,3-BPG found in the structure (see figure) The function: Stabilize the T-Form and important in the delivery of oxygen to peripheral	-





Hemoglobin Function

- Hemoglobin carries oxygen from the lungs to tissues (The major function)
- Hemoglobin carries carbon dioxide (In form of carbaminohemoglobin) and protons from tissues back to the lung
- Normal level of hemoglobin (g/dL)
  - Male: 14-16 (g/dL)
  - Female: 13-15 (g/dL) (Menstrual cycle is the reason that female has less Hemoglobin than Male)



Factors Affecting Oxygen Binding

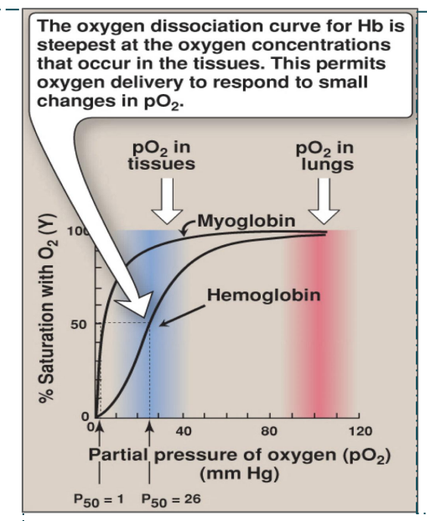
The ability of hemoglobin to reversibly bind to oxygen is due to four **allosteric effectors:**
(Allosteric factors are going to bind to hemoglobin and modify O2 binding capacity):

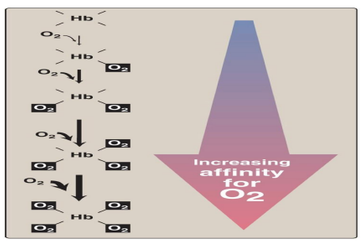
1. pO_2 (partial oxygen pressure) through heme–heme interactions (How much O_2 are present?)
2. pH of the environment (How much H are present?)
3. pCO_2 (partial CO_2 pressure)
4. Availability of 2,3-bisphosphoglycerate (also known as 2,3- diphosphoglycerate) Important to stabilize the deoxyhemoglobin

Allosteric effectors: Their interaction at one site on the hemoglobin molecule affects the binding of oxygen to heme groups at other sites on the molecule.

Oxygen Dissociation Curve

- The curve is **sigmoidal** in shape (S shaped curve), It indicates cooperation of subunits in O2 binding.
- The shape of the curve has a **lag** phase initially and a **steep** phase which indicates the presence of cooperative binding.
- Cooperative binding means the binding of the first molecule facilitate the binding for the second molecule.
- 4 molecules of O_2 binding to hemoglobin = 100% saturated, but if 3 molecules of O_2 binding to hemoglobin = 75% saturated and so on
- **Heme-heme interaction**: Binding of O_2 to one heme group increases O_2 affinity of others (means that the binding of an oxygen molecule at one heme group increases the oxygen affinity of the remaining heme groups in the same hemoglobin).
- Although it is more difficult for the first oxygen molecule to bind to hemoglobin, the subsequent binding of oxygen occurs with higher affinity, as shown by the steep upward curve in the region near 20–30 mmHg





Heme-Heme Interaction

What is P_{50}?

- P_{50} (mmHg): it is the partial O_2 pressure at which Hb is 50% saturated with O_2.
- It indicates affinity of Hb to O_2.
  - **High** affinity → **slow** unloading of O_2
  - **Low** affinity → **fast** unloading of O_2

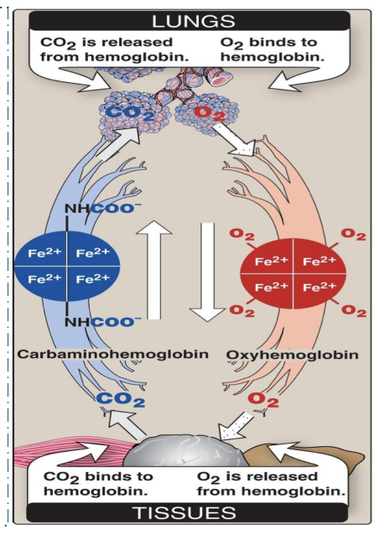
لأن لما يكون عندنا الـ PO_2 قليل فنحتاج أن الهيموقلوبين يعطينا الأوكسجين اللي معه

When lung partial pressure of O_2 is 100 mmHg → Hb saturation is 100%

When tissue partial pressure of O_2 is 40 mmHg → Hb saturation reduces, hence O_2 is delivered to tissue.

يعني باختصار اللي يحتاج تعرفونه من هالصورة أن الـ Hb ياخذ الأوكسجين من الـ Lungs ويصير اسمه (Oxyhemoglobin) بعدها يروح للـ Tissues وهناك يُفرز الـ Protons اللي راح يقلل الـ Affinity حقت الـ Hb للـ O_2 وراح يخليه يترك الأوكسجين وفي نفس الوقت راح تكون الـ Tissues أفرزت الـ CO_2 وحنا قلنا الـ Hb حاليًا ترك الأوكسجين فهو فاضي! يقوم وياخذ الـ CO_2 ويصير اسمه (Carbaminohemoglobin) ويروح يوديه للـ Lungs وبعدها يتركه ويأخذ أوكسجين وتستمر الدوامة

In lung: ↑pO_2 → ↑ Hb binding → high affinity → slow unloading of O_2
In tissue: ↓pO_2 → ↓ Hb binding → low affinity → fast unloading of O_2



For better understanding:

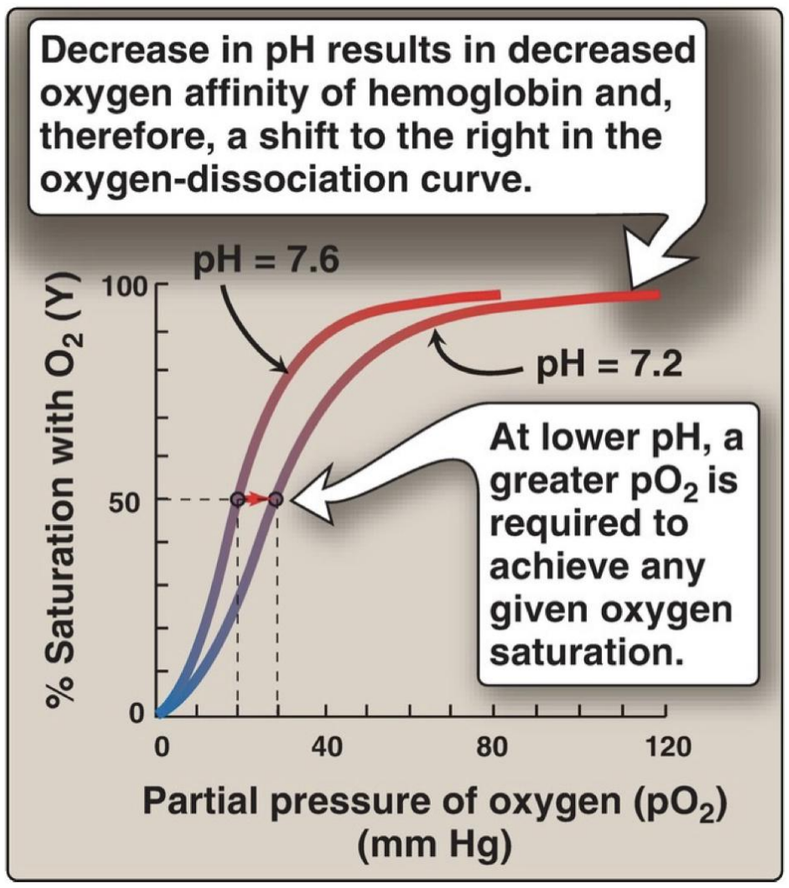
Helpful video Helpful video

The ability of hemoglobin to reversibly bind oxygen is affected by the pO_2 (through heme–heme interactions as described above), the pH of the environment, the partial pressure of carbon dioxide (pCO_2) and the availability of 2,3-bisphosphoglycerate. These are collectively called allosteric ("other site") effectors, because their interaction at one site on the hemoglobin molecule affects the binding of oxygen to heme groups at other sites on the molecule.

oxygen-dissociation curve for hemoglobin is sigmoidal in shape (see Figure 3.5), indicating that the subunits cooperate in binding oxygen. Cooperative binding of oxygen by the four subunits of hemoglobin means that the binding of an oxygen molecule at one heme group increases the oxygen affinity of the remaining heme groups in the same hemoglobin tetramer (Figure 3.6). This effect is referred to as heme–heme interaction (see below). Although it is more difficult for the first oxygen molecule to bind to hemoglobin, the subsequent binding of oxygen occurs with high affinity, as shown by the steep upward curve in the region near 20–30 mmHg (see Figure 3.5).

The Bohr Effect

The effect of pH and PCO_2 on affinity of hemoglobin to oxygen



It is the shift of the ODC "oxygen dissociation curve" to **the right** in response to an increase in pCO_2 or a decrease in pH.

(pH decreases because the tissues are actively metabolizing and releasing CO_2, and CO_2 reacts with water to make carbonic acid by help of carbonic anhydrase, after that the carbonic acid dissociates to bicarbonate and hydrogen)

It describes the Effect of pH (Hydrogen ions) and pCO_2 on:

- Oxygenation of Hb in the lungs.
- Deoxygenation in tissues.

- Affinity is showing by saturations, if the curve shifts to the right that means the affinity is reduced and lead to unload (release) the oxygen

In simple the Bohr effect shows how the production of CO_2 leads to increase the production of protons (Hydrogens) that lowers the affinity between the Hb and O_2 (so increase the release of O_2 to the tissues)

In the Lung

Protons are released and react with HCO_3^- to form CO_2 gas: $HCO_3^- + H^+ \longrightarrow CO_2 + H_2O$

PO_2 is high, so the Hb that leave the lung is bind to O_2 and we call it Oxyhemoglobin.

- The proton-poor Hb now (**in the lungs**) has greater affinity for O_2 .
- The Bohr effect removes insoluble CO_2 from blood stream, and Produces soluble bicarbonate.

In the Tissues

- **Tissues** have lower pH (acidic) than lungs due to proton generation (two reactions):

$CO_2 + H_2O \longleftrightarrow H_2CO_3 \longleftrightarrow HCO_3^- + H+$

Tissues are releasing CO_2 which bind to Hb and make it become (Carbaminohemoglobin)

- Protons reduce O_2 affinity of Hb causing easier O_2 release (unload) into the tissues.
- The free Hb binds to two protons.

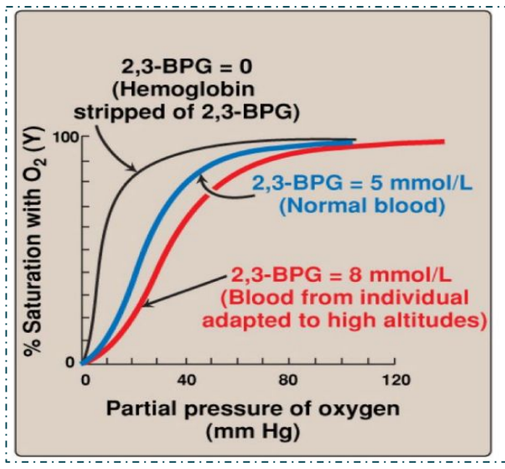
Availability of 2,3-Bisphosphoglycerate

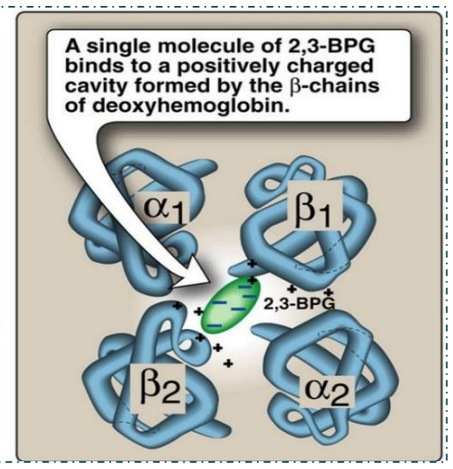
It is molecule produces in RBCs during the glycolytic pathway

The function: it goes and sets between the two beta chains and stabilizes the deoxyhemoglobin structure (Facilitates the releasing of O2)

In the Lung — When oxygen binds to Hb, BPG is released.

In the tissues — BPG Binds to deoxy-hb and stabilizes the T-form.





When does 2,3-BPG become zero?

When the blood stored for long time, that lead to decrease the amount of 2,3-BPG..

And if the patient takes multiple transfusions from that blood it will be not rich in 2,3-BPG that leads to increase the affinity between O_2 and Hb (Called O_2 trapped) so the O_2 will not deliver to the tissues..

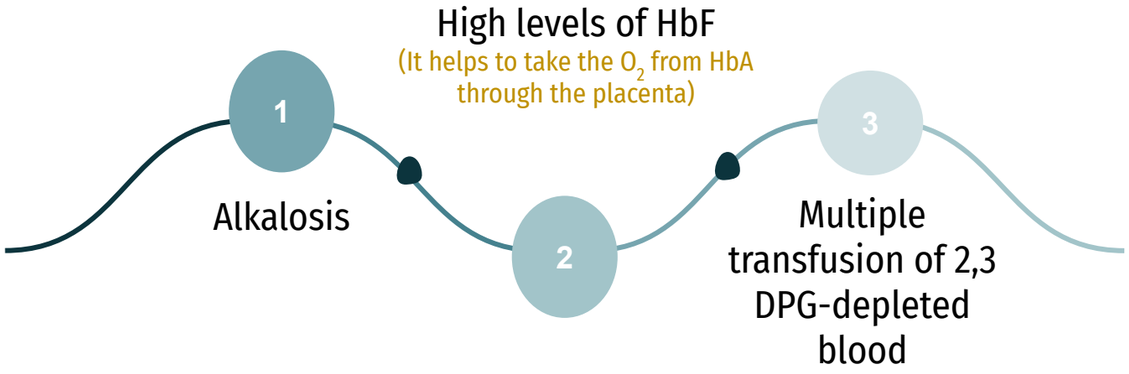
However the patient needs 12-24 hours to makeup 2,3-BPG then start working

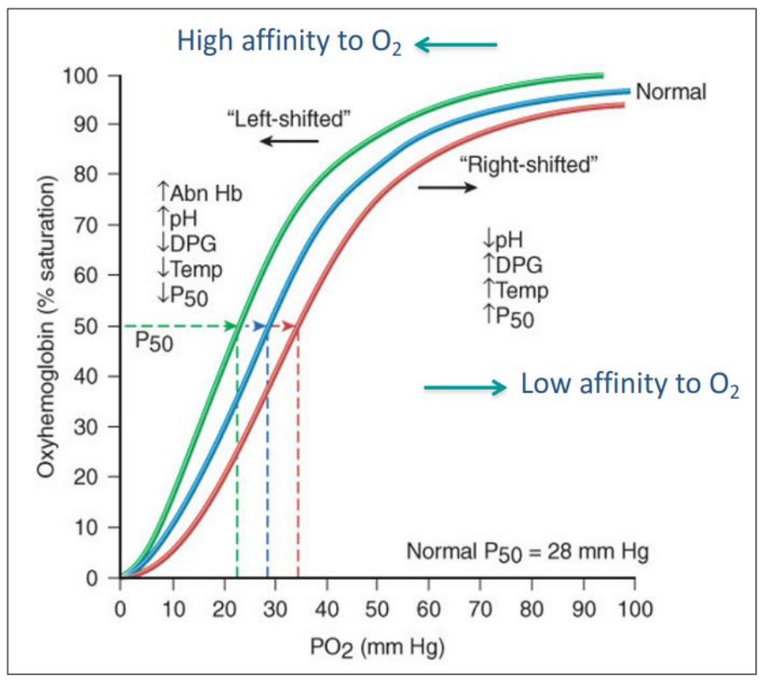
At high altitude Or COPD → **↑RBC number** To carry more hemoglobin → **↑Hb conc** → **↑2,3 BPG**

High altitude and O2 affinity

In hypoxia and high altitude: → **2,3 BPG levels↑** → **↓O_2 affinity of Hb** → **↑O_2 delivery to tissues**

High O_2 affinity is due to:



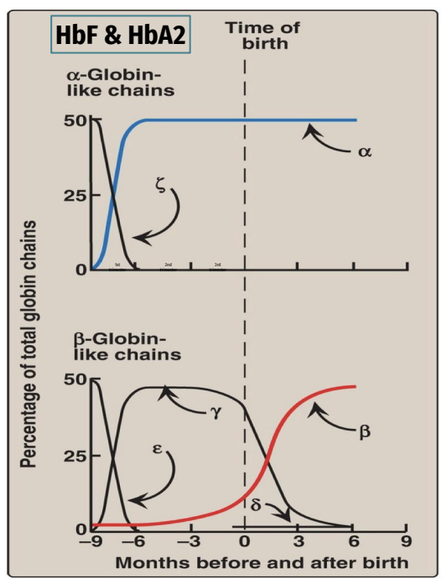


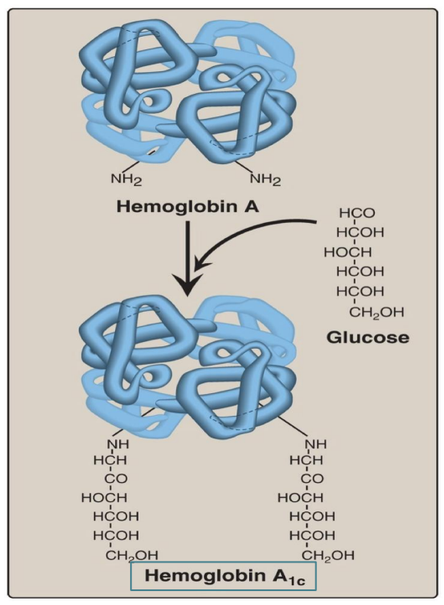
مهمة التفاصيل اللي داخل الصورة ولازم تعرفون أيش الأشياء اللي تخلي الـ curve يصير له Shift لليمين وأيش اللي تسويه له shift لليسار لأن ممكن يجي عليهم أسئلة بناءًا على كلام د.سمبل

Other hemoglobin forms

Types	HbF	HbA_2	HbA1c
Structure (All Tetramer)	Tetramer with two **α** and two (gamma) **γ** chains	Composed of two **α** and two (delta) **δ** globin chains	Two **α** and two **β** -Glucose
Found	Major hemoglobin found in the fetus and newborn.	Appears shortly before birth.	High in patients with diabetes mellitus.
Deference	• Higher affinity for O_2 than HbA • Transfers O_2 from maternal to fetal circulation across placenta	Constitutes ~2% of total Hb	• it's HbA undergoes non-enzymatic glycosylation In other word the reaction will happen by Presence of HbA with glucose in blood. • Glycosylation depends on plasma glucose levels

In the first curve you just need to know in the 1st trimester (the first 3 months of pregnancy) the major form of globins chains are zeta chains not the alpha chains, but in the 2nd trimester the alpha chains increase and becomes the major form.
In the second curve the gamma chains start to appear in the second trimester and becomes the major form until the birth, after the birth the beta chains become the major form.





Summary

Hemoglobin

Function	1. Carries oxygen from the lungs to tissue 2. Carries carbon dioxide from tissues back to the lungs	
Types	**Normal** 1. HbA : 2α. 2B 2. HbA2: 2α.2δ 3. HbF: 2α .2γ 4. HbA1c: 2α. 2B-glucose	**Abnormal** 1. Carboxy Hb 2. Met Hb 3. Sulf Hb
Normal level	Males (14-16) Females (13-15)	
Factors affecting O_2 binding	1. pO_2 (partial oxygen pressure) 2. pH of the environment 3. pCO_2 (partial carbon dioxide pressure) 4. availability of 2,3-bisphosphoglycerate	
The bohr effect is	the shift of the oxygen dissociation curve to the right in response to an increase in pCO_2 or a decrease in pH	
If the curve shifts to the left	1. High affinity to O_2 2. increase in abnormal Hb & pH 3. decrease in DPG, temperature & p_{50}	
If the curve shifts to the right	1. low affinity to O_2 2. increase in DPG, temperature & p_{50} 3. decrease in pH	

MCQs

1- Which of the following is NOT bound to the heme group of hemoglobin?			
A- Histidine	B- Nitrogen	C- Oxygen	D- Sulfur
2- Which of the following is a feature of R-form of hemoglobin?			
A- movement of dimer is constrained	B- Low oxygen affinity	C- Abundant 2,3-BPG	D- Oxygenated form of Hb
3- Which of the following is NOT an allosteric effector?			
A- pH of environment	B- pO_2	C- pCO	D- Availability of 2,3- BPG
4- Which of the following is irreversible?			
A-Sulf Hb	B- Met Hb	C-Carboxy Hb	D-None of the above
5- : HbA_2 is composed of ?			
A- Two α and two δ globin chains.	B- Two α and two γ chains.	C-Four α globin chains	D- Two α and two β chains
6-Ali is patient with hypoxia went to visit his uncle in Abha, what do you expect to find?			
A- BPG ↓	B- O_2 delivery to tissues ↓	C-O_2 affinity ↓	D-A&C

Answers key

1-D 2- D 3- C 4- A 5- A 6- C

SAQs

1- What are the factors affecting O_2 binding?

- pO_2
- pCO_2
- pH of the environment
- Availability of 2,3-Bisphosphoglycerate

2- Enumerate the factors that cause Left shift of the Oxygen Dissociation Curve

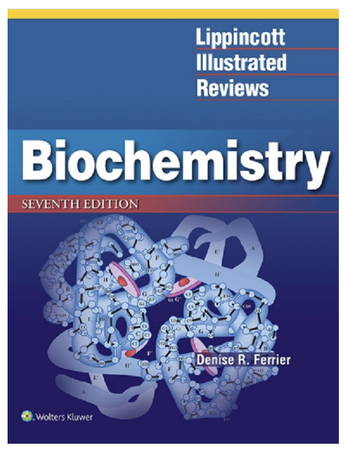
- Inc. pH, Alkalosis, dec. Protons
- Dec. Temperature
- Dec. P_{50}
- Dec. 2,3-BPG
- Abnormal Hb
- High levels of HbF

3- What's the function of Hemoglobin?

- Carries oxygen from the lungs to tissues and carbon dioxide from tissues back to the lungs

Resources

Click on the book to download the resource



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Special thanks to Fahad AlAjmi for designing our team's logo.