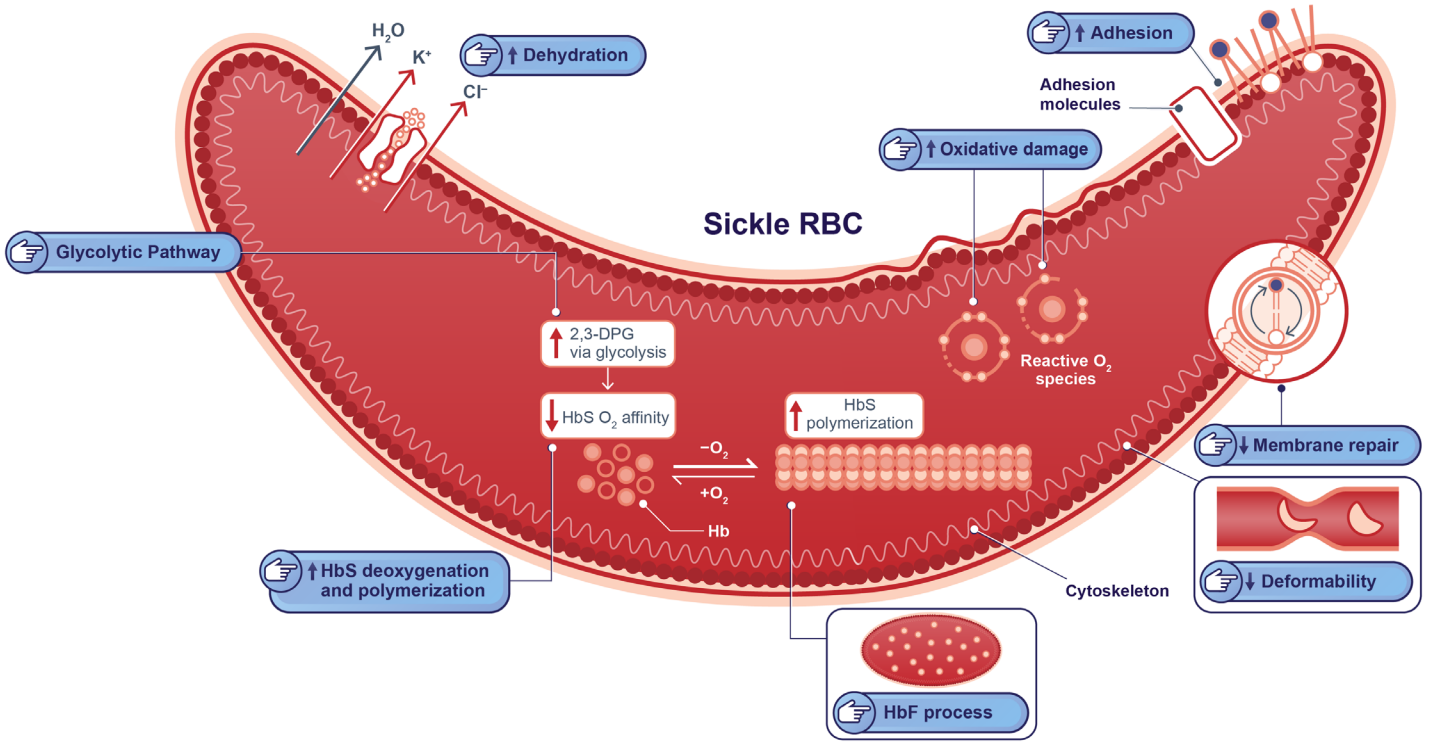
Red Blood Cell Health

Elements of SCD Pathophysiology

Overview: Elements of SCD Pathophysiology

- Sickled RBCs have higher levels of 2,3-DPG and lower levels of ATP than healthy RBCs[1,2]
- Elevated levels of HbF have been associated with improved RBC lifespan in SCD[3]



Impaired RBC health in SCD leads to hemolysis, vaso-occlusion, and reduced RBC lifespan[1,4]

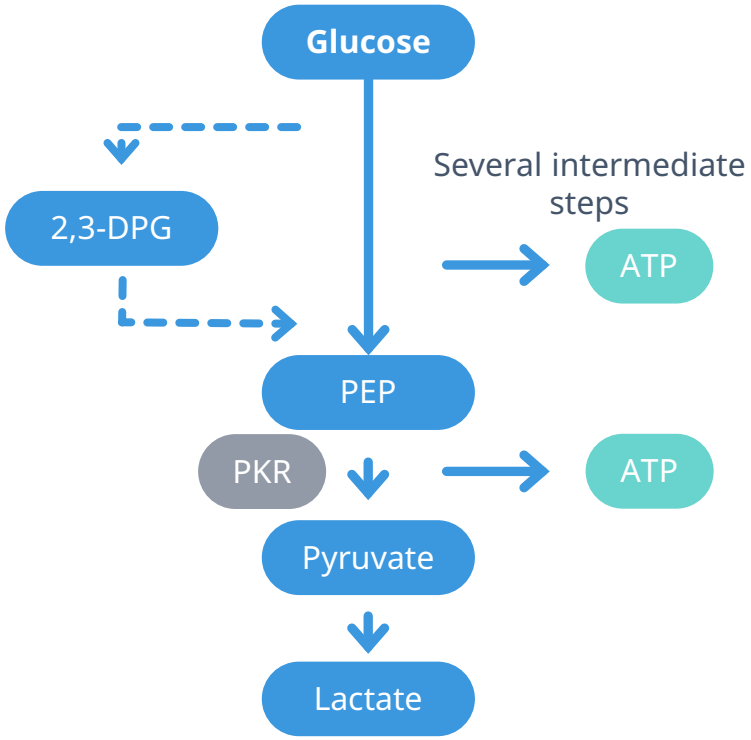
2,3-DPG, 2,3-diphosphoglycerate; ATP, adenosine triphosphate; HbF, fetal hemoglobin; HbS, sickle hemoglobin; RBC, red blood cell; SCD, sickle cell disease.

1. Kato GJ, et al. *Nat Rev Dis Primers*. 2018;4:18010. 2. Banerjee T, Kuypers FA. *Br J Haematol*. 2004;124(3):391-402. 3. Franco RS, et al. *Blood*. 2006;108(3):1073-1076. 4. Vona R, et al. *Antioxidants (Basel)*. 2021;10(2):296.

HbS polymerization results in sickled RBCs[1]

- SCD is a genetic disorder characterized by abnormal hemoglobin (HbS) that polymerizes under conditions of low oxygen tension[1]
- 2,3-diphosphoglycerate (2,3-DPG) is a byproduct of glycolysis and is a negative regulator of hemoglobin-oxygen affinity[2,3]
- HbS exists in a relaxed state (R-state) or a tense state (T-state), which impacts oxygen affinity[1,4]
  - In the R-state, Hb has a high affinity for oxygen and is associated with low levels of 2,3-DPG[4]
  - In the T-state, Hb has a low affinity for oxygen and is associated with higher levels of 2,3-DPG[4]

Glycolysis pathway[2]



The glycolytic pathway is a source of ATP generation in RBCs for membrane repair, hydration, and deformability[2,5]

ATP, adenosine triphosphate; Hb, hemoglobin; HbS, sickle hemoglobin; PEP, phosphoenolpyruvate; PKR, pyruvate kinase; RBC, red blood cell; SCD, sickle cell disease.

1. Kato GJ, et al. *Nat Rev Dis Primers*. 2018;4:18010. 2. van Wijk R, van Solinge WW. *Blood*. 2005;106(13):4034-4042. 3. Eaton WA, Bunn HF. *Blood*. 2017;129(20):2719-2726. 4. Oder E, et al. *Br J Haematol*. 2016;175(1):24-30. 5. Huisjes R, et al. *Front Physiol*. 2018;9:656.

Back to overview

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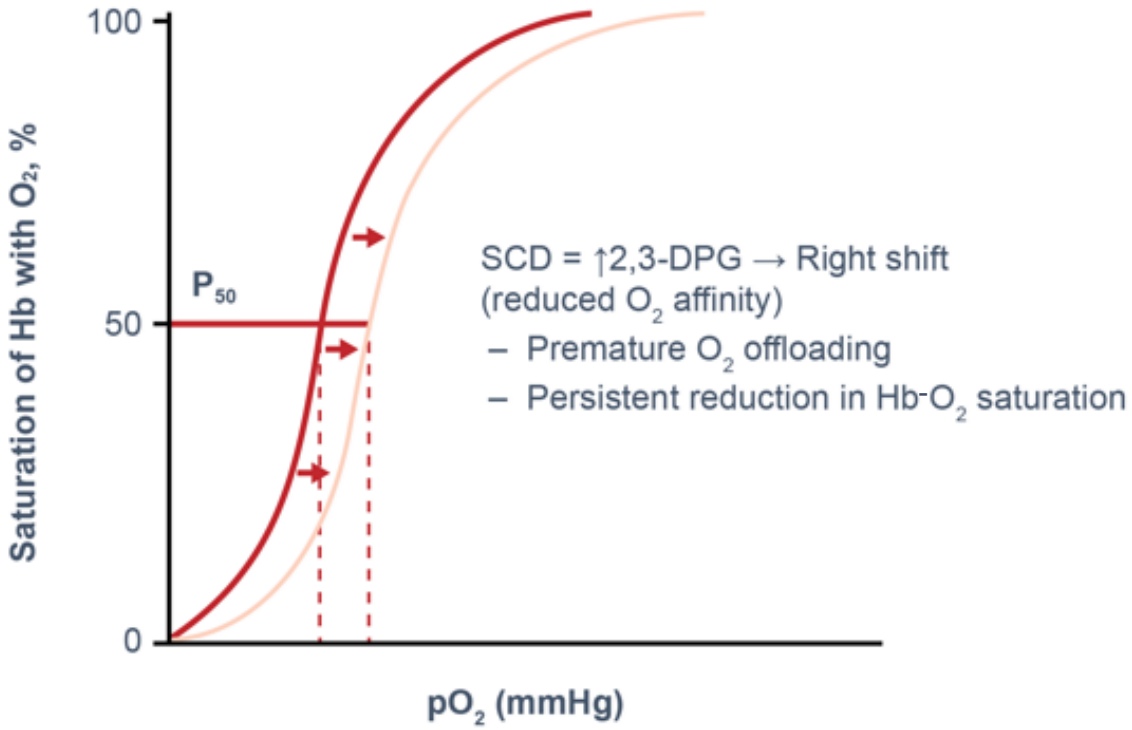
2,3-DPG is the major allosteric effector of Hb-O_2 affinity[1]

O_2-Hb dissociation curve[2]



- 2,3-DPG regulates the ability of RBCs to carry and deliver O_2[2]
- Under conditions of low O_2 tension, 2,3-DPG binds to the cleft between β subunits, stabilizing HbS in the tense state[1]
- Increased 2,3-DPG
  - Stabilizes HbS polymers by decreasing solubility[1]
  - Decreases intracellular pH, which reduces HbS solubility and O_2 affinity (the Bohr effect)[1,2]
- Ultimately, increased 2,3-DPG potentiates HbS polymerization[1]

Fetal Hb (HbF) does not interact with 2,3-DPG, resulting in a high affinity for O_2[3]

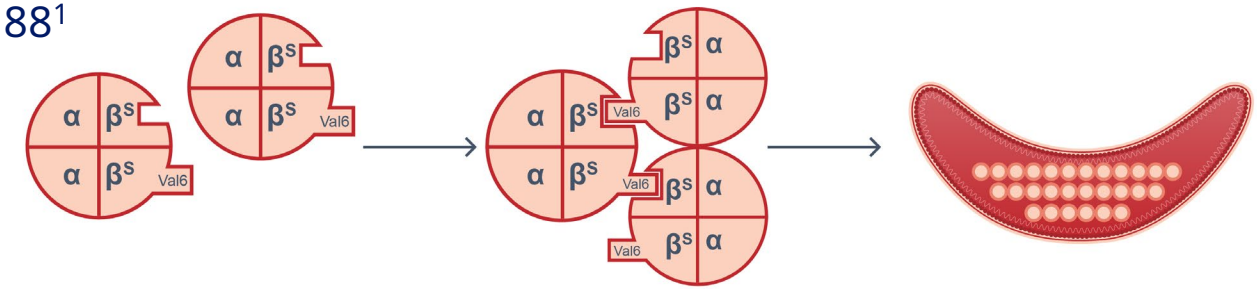
2,3-DPG, 2,3-diphosphoglycerate; Hb, hemoglobin; HbS, sickle hemoglobin; RBC, red blood cell; SCD, sickle cell disease.

1. Eaton WA, Bunn HF. *Blood*. 2017;129(20):2719-2726. 2. MacDonald R. *Anaesthesia*. 1977;32(6):544-553. 3. Kaufman DP, et al. Physiology, fetal hemoglobin. In: *StatPearls*. StatPearls Publishing. Updated March 20, 2023. Accessed May 24, 2023. http://www.ncbi.nlm.nih.gov/books/NBK500011/

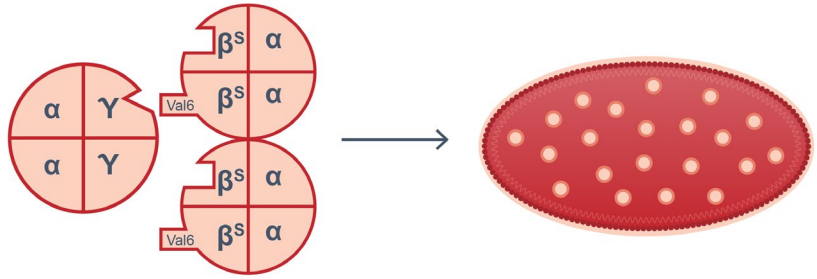
Back to overview

HbF prevents polymerization of HbS

- In SCD, HbS tetramers polymerize into long strands, **stabilized by hydrophobic interactions** between strands at β positions 6 and 85 to 88[1]



- HbF has a γ-globin with Gln at position 87 (vs Thr), which **weakens the hydrophobic interaction** between strands, blocking polymerization and preventing the formation of long polymer strands found in SCD[1]
- Additionally, HbF has a decreased affinity for 2,3-DPG, which further prevents HbS polymerization[2,3]



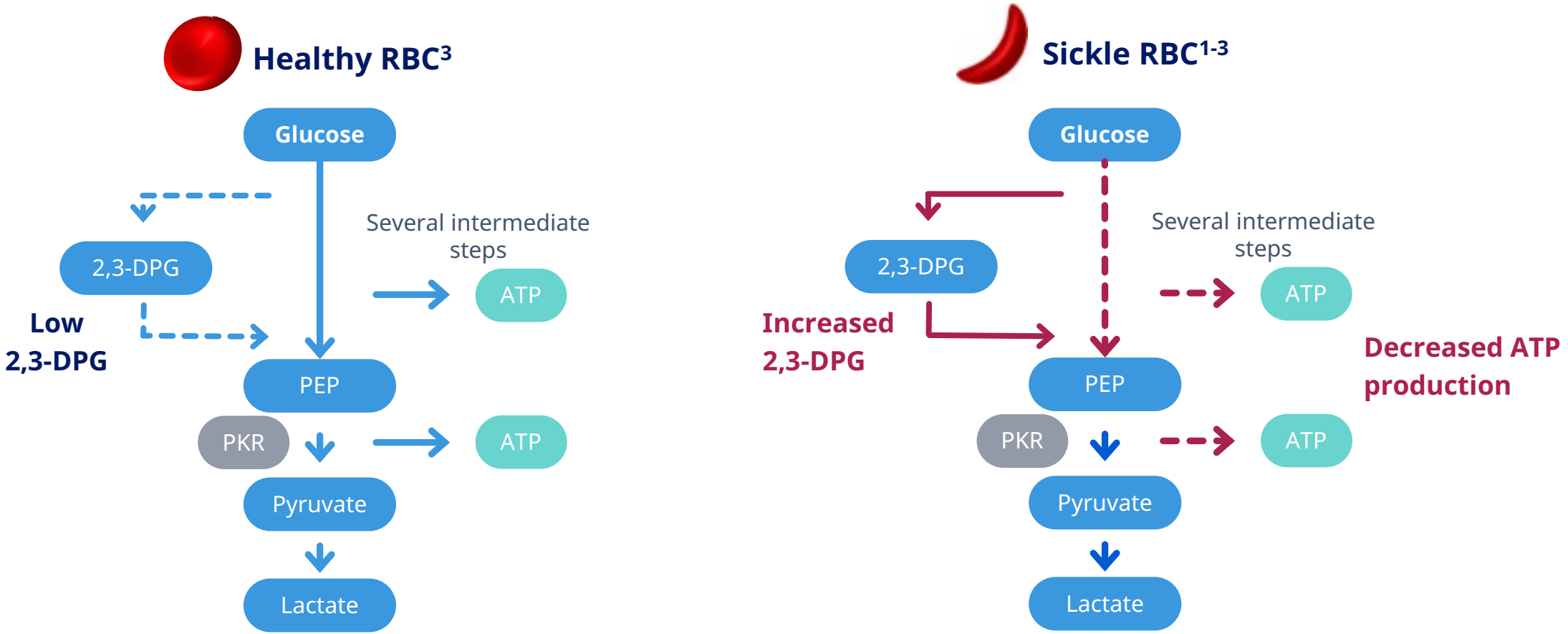
Even small increases in HbF have shown to improve RBC health and clinical outcomes[4]

2,3-DPG, 2,3-diphosphoglycerate; Gln, glutamine; HbF, fetal hemoglobin; HbS, sickle hemoglobin; SCD, sickle cell disease; Thr, threonine.

1. Lettre G, Bauer DE. *Lancet*. 2016;387(10037):2554-2564. 2. Kaufman DP, et al. Physiology, fetal hemoglobin. In: *StatPearls*. StatPearls Publishing. Updated March 20, 2023. Accessed May 24, 2023. http://www.ncbi.nlm.nih.gov/books/NBK500011/ 3. Eaton WA, Bunn HF. *Blood*. 2017;129(20):2719-2726. 4. Steinberg MH. *Blood*. 2020;136(21):2392-2400.

Back to overview

Sickle RBCs have higher levels of 2,3-DPG and lower levels of ATP than healthy RBCs[1,2]



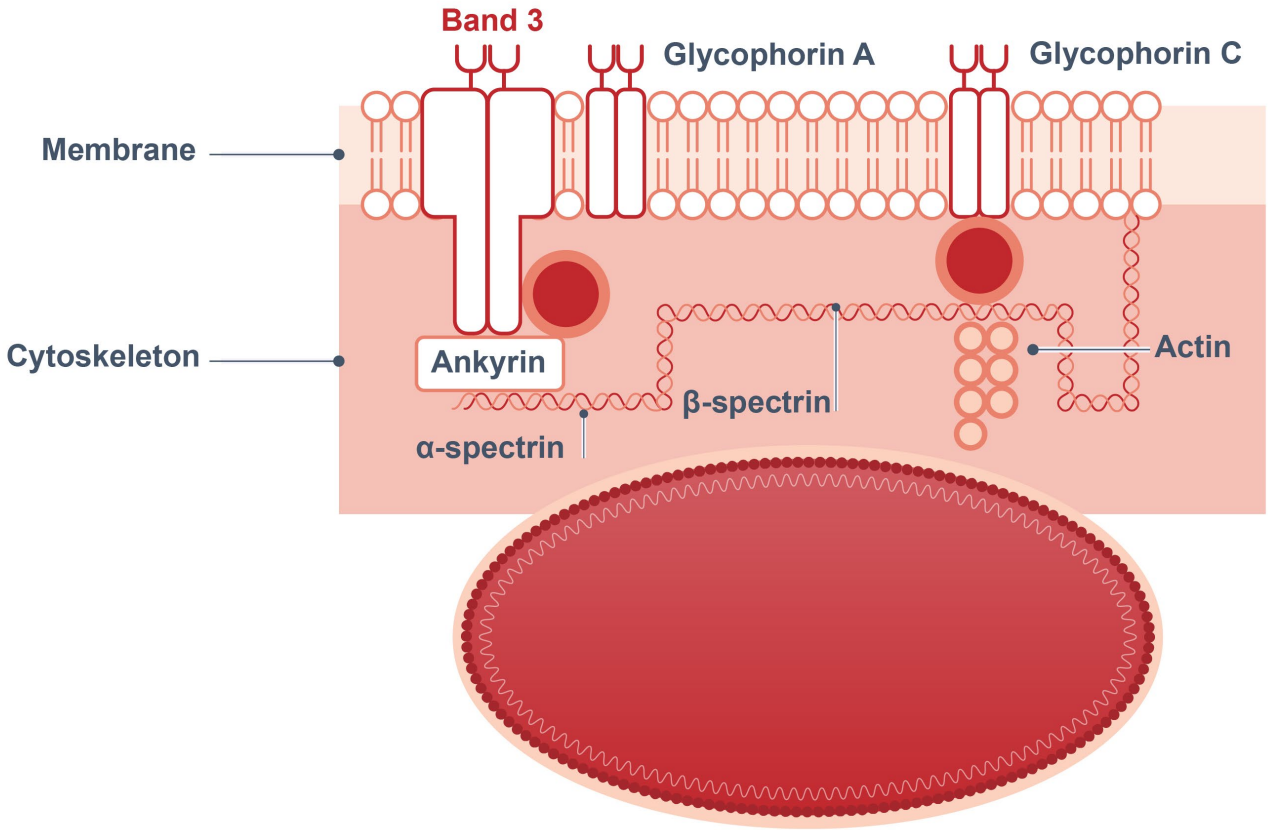
2,3-DPG, 2,3-diphosphoglycerate; ATP, adenosine triphosphate; PEP, phosphoenolpyruvate; PKR, pyruvate kinase; RBC, red blood cell.

1. Kato GJ, et al. *Nat Rev Dis Primers*. 2018;4:18010. 2. Banerjee T, Kuypers FA. *Br J Haematol*. 2004;124(3):391-402. 3. van Wijk R, van Solinge WW. *Blood*. 2005;106(13):4034-4042.

Back to overview

Sickle RBCs have impaired deformability[1]

Healthy RBCs need to deform to traverse vessels under shear stress without rupture.[1]



- Sickle RBCs have impaired deformability and become stiff due to polymerization[1]
- RBC deformability is regulated and maintained by interactions between membrane and cytoskeletal proteins[1]
- **Low levels of ATP in sickle RBCs, along with HbS polymerization, result in impaired deformability[1,2]**

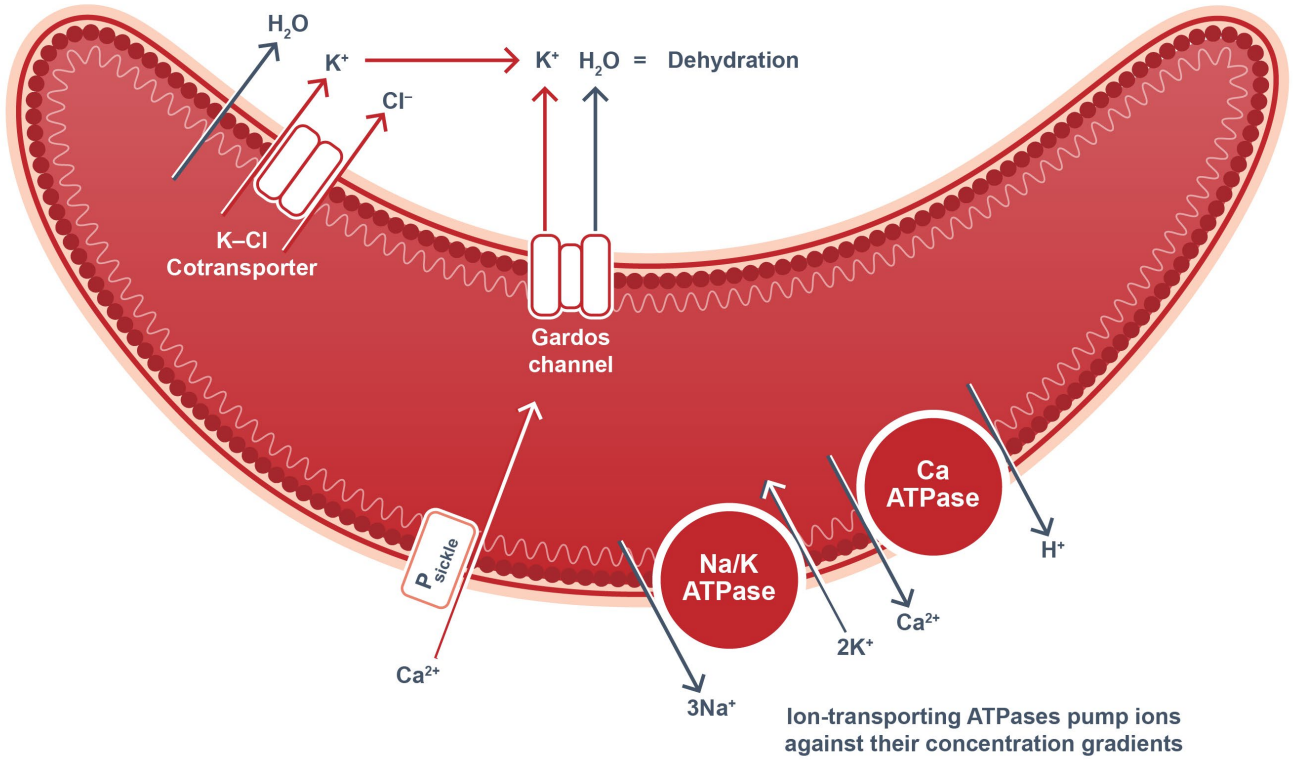
ATP, adenosine triphosphate; HbS, sickle hemoglobin; RBC, red blood cell.

1. Huisjes R, et al. *Front Physiol*. 2018;9:656. 2. Mohanty JG, et al. *Front Physiol*. 2014;5:84.

Back to overview

Impaired ion hemostasis causes sickle RBC dehydration[1-3]

Healthy RBCs maintain significant ion gradients via the action of ATP-dependent cation pumps.[2]



- Sickle RBCs leak cations as a result of sickling[1]
- Divalent cation permeability is particularly important as increased intracellular Ca^{2+} and decreased Mg^{2+} stimulate K^+ efflux[2,3]
  - As intracellular K^+ concentration decreases, water flows out of the RBC[2,3]
- **Dehydrated RBCs sickle more rapidly due to the higher concentration of HbS[4]**
- Homeostasis can be restored/maintained only by the expenditure of ATP to pump ions against their concentration gradients[2]

ATP, adenosine triphosphate; ATPase, adenosine triphosphatase; HbS, sickle hemoglobin; RBC, red blood cell.

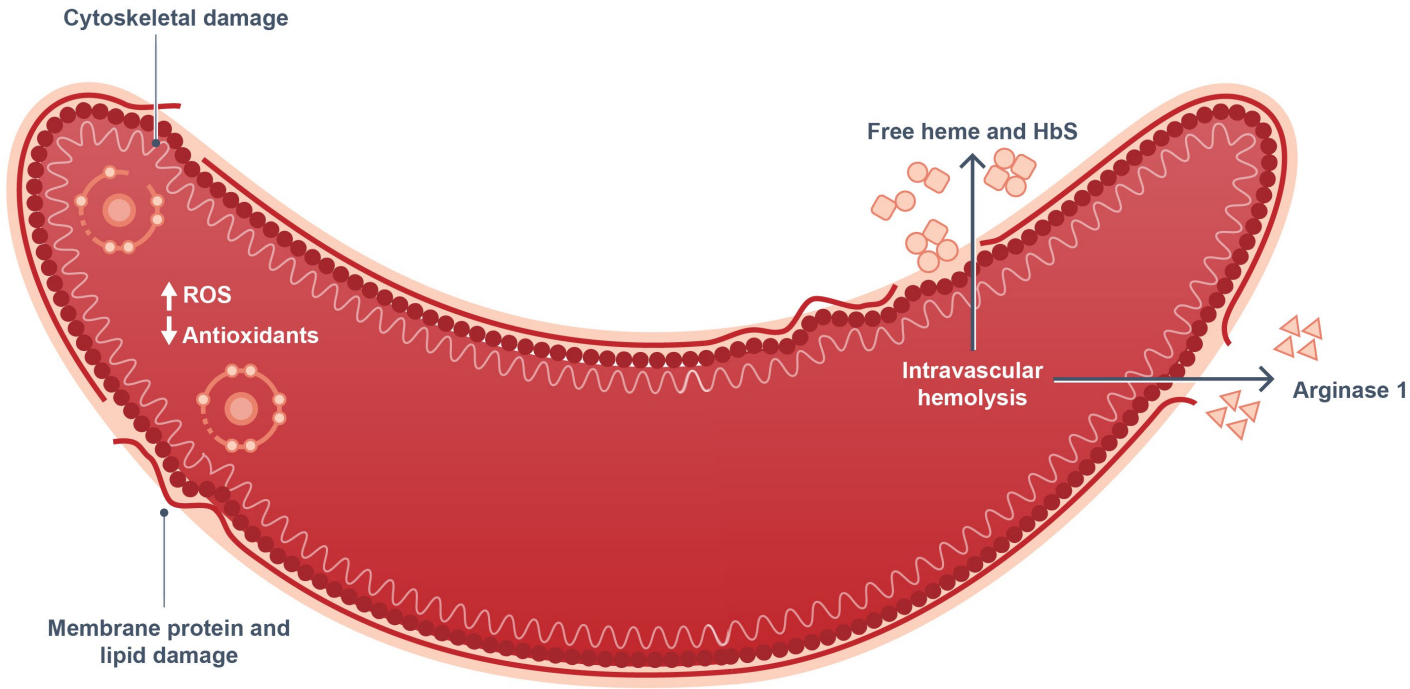
1. Lew VL, Bookchin RM. *Physiol Rev*. 2005;85(1):179-200. 2. Huisjes R, et al. *Front Physiol*. 2018;9:656. 3. Brugnara C, et al. *J Clin Invest*. 1996;97(5):1227-1234. 4. Kato GJ, et al. *Nat Rev Dis Primers*. 2018;4:18010.

Back to overview

Sickle RBCs have decreased antioxidant capacity[1]

Healthy RBCs contain helpful enzymes that serve as antioxidants to scavenge ROS.[2]

- ROS are harmful to membrane proteins and can cause lipid damage and cytoskeletal defects[1]
- **Antioxidant enzymes, including glutathione reductase and superoxide dismutase, are ATP-dependent[1]**
- Lack of ATP results in decreased enzymatic activity that causes increased oxidative damage to the membrane and cytoskeleton, leading to increased intravascular hemolysis[1,3]



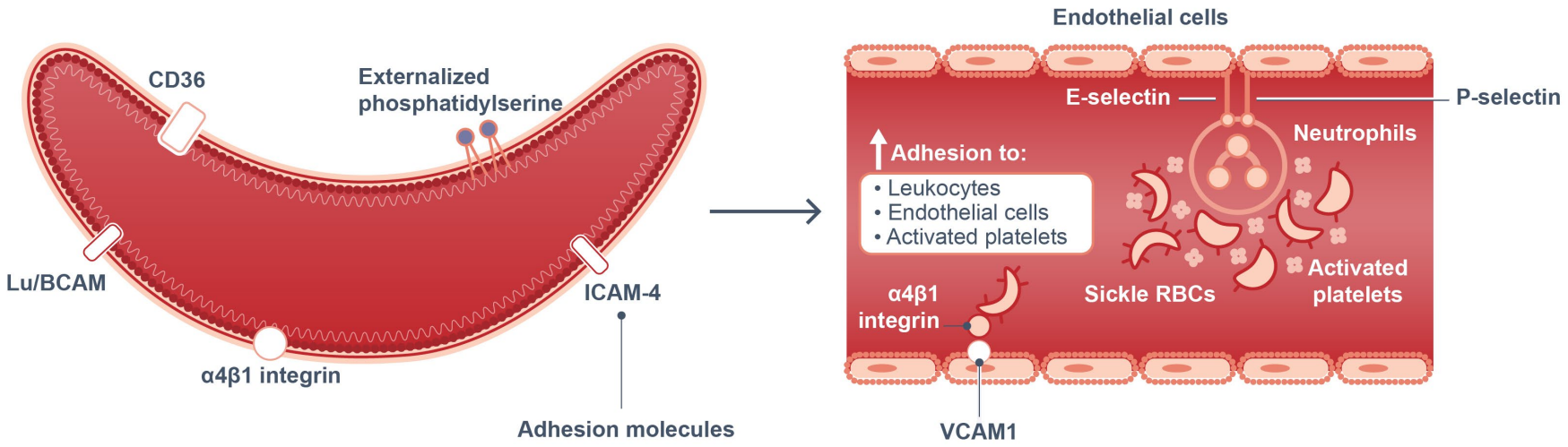
ATP, adenosine triphosphate; RBC, red blood cell; ROS, reactive oxygen species.

1. Vona R, et al. *Antioxidants (Basel)*. 2021;10(2)296. 2. Mohanty JG, et al. *Front Physiol*. 2014;5:84. 3. Kato GJ, et al. *Nat Rev Dis Primers*. 2018;4:18010.

Back to overview

Sickle RBCs exhibit increased adhesion[1]

Healthy RBCs express amino phospholipids, such as phosphatidylserine (PS), on the inner leaflet of the membrane and do not aggregate.[2]



- Sickle RBCs exhibit increased adhesion and overexpress adhesion molecules, such as α4β1 integrin and Lu/BCAM. This may lead to endothelial activation, thereby causing vaso-occlusion[1,3,4]
- Sickling induces reorientation of PS from the inner to the outer surface of RBCs, making RBCs stickier and leading to premature clearance of sickled RBCs by macrophages and vaso-occlusion[5]
- **PS can be reoriented to the inner leaflet by an ATP-dependent translocase[2,4]**

ATP, adenosine triphosphate; CD, cluster of differentiation; ICAM-4, intracellular adhesion molecule 4; Lu/BCAM, Lutheran/basal cell adhesion molecule; RBC, red blood cell; VCAM1, vascular cell adhesion molecule 1.

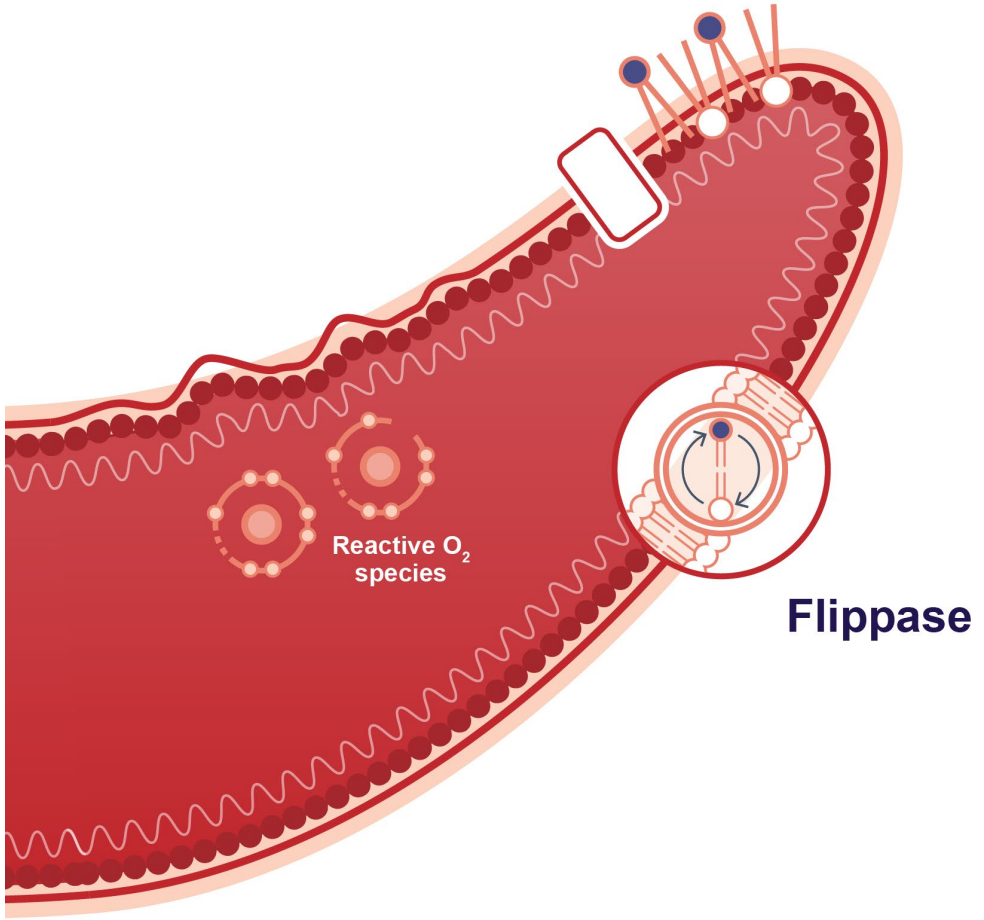
1. Kato GJ, et al. *Nat Rev Dis Primers*. 2018;4:18010. 2. Weiss E, et al. *Anemia*. 2011;2011:379894. 3. McMahon TJ. *Front Physiol*. 2019;10:1417. 4. Hannemann A, et al. *Br J Haematol*. 2018;182(4):567-578. 5. Setty BN, et al. *Blood*. 2002;99(5):1564-1571.

Back to overview

Low levels of ATP result in impaired membrane repair[1]

Healthy RBCs maintain functional integrity of key cytoskeletal and membrane components, which are dependent on ATP.[2]

- Translocase (also known as *flippase*) reorients PS to the inner leaflet[1]
- **Flippase requires ATP to repair membranes in various hemoglobinopathies, including SCD[3]**
- Inhibition of flippase can be triggered by oxidant stress, increased intracellular Ca^{2+}, and the activity of protein kinases[3]



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Back to overview