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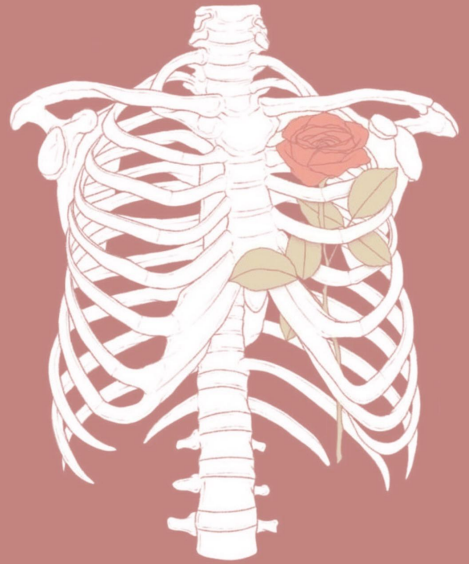
Creatine Metabolism



Color index :

Main text

IMPORTANT

Extra Info

Drs Notes

Muskuloskeletal Block - Biochemistry Team

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MED439

Objectives:

- Study the importance of creatine in muscle as a storage form of energy.
- Understand the biosynthesis of creatine.
- Study the process of creatine degradation and formation of creatinine as an end product.
- Understand the clinical importance of creatinine as a sensitive indicator of kidney function.
- Study the structure, function, types, and biosynthesis of collagen
- Understand different diseases associated with collagen.

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Creatine Biosynthesis

- Three amino acids are required:
  1. Glycine.
  2. Arginine.
  3. Methionine (as S-Adenosylmethionine).
     - S-Adenosylmethionine (SAM)= ATP + Methionine.

- One amino acid is produced:
  - Ornithine.

- **Site of biosynthesis:**

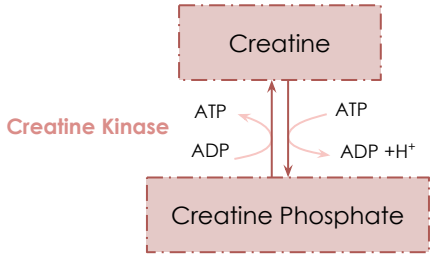
Through plasma

Step 1: Kidney → **Step 2:** Liver

- Half of the things are done in the kidney.
- Guanidinoacetate is produced

- Then it will move to the liver, so the final biosynthesis is in the liver.
- If we ask where is creatine phosphate synthesized? It synthesized in the liver.
- Creatine is produced.

- **Distribution of Creatine:**
- Formed in the liver, then transported from liver to other tissues.
- 98% of body creatine is in skeletal and heart muscles. where is Creatine Kinase? It's in the muscles
- In skeletal muscle Creatine is converted to high-energy source Creatine phosphate by the help of **Creatine Kinase** (phosphocreatine of Creatine).
- ATP <u>In</u>.
- ADP <u>Out</u>.
- Creatine phosphate (another form of creatine) is reserved in muscles as a main storage form of energy.
- During exercise, ATP is consumed rapidly into ADP, now we know that glycolysis does that, But not this fast and specifically speaking, during the first seconds of muscle contraction Energy is supplied by Creatine Phosphate when converting it back to Creatine.



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Creatine Biosynthesis

1 Arginine + Glycine —(**Amidinotransferase**)→ Guanidinoacetate

- Ornithine Out. It's a byproduct and non proteinogenic which means it's not encoded or found in the genetic code
- Transformation of the amidino group from arginine to glycine to form = Guanidinoacetate by the help of **Amidinotransferase** enzyme which will go through the blood to the liver.

2 Guanidinoacetate —(**Methyltransferase**)→ Creatine

- S-Adenosylmethionine (SAM) In.
- S-Adenosylhomocysteine (SAH) Out.
- Transformation of a methyl group from SAM and adds it to Guanidinoacetate that will convert SAM into SAH to form Creatine by the help of **Methyltransferase** enzyme.
- Creatine will be transported to other tissues.
- Guanidinoacetate present in the blood, liver and kidney, When it's in the liver it will be converted to creatine.

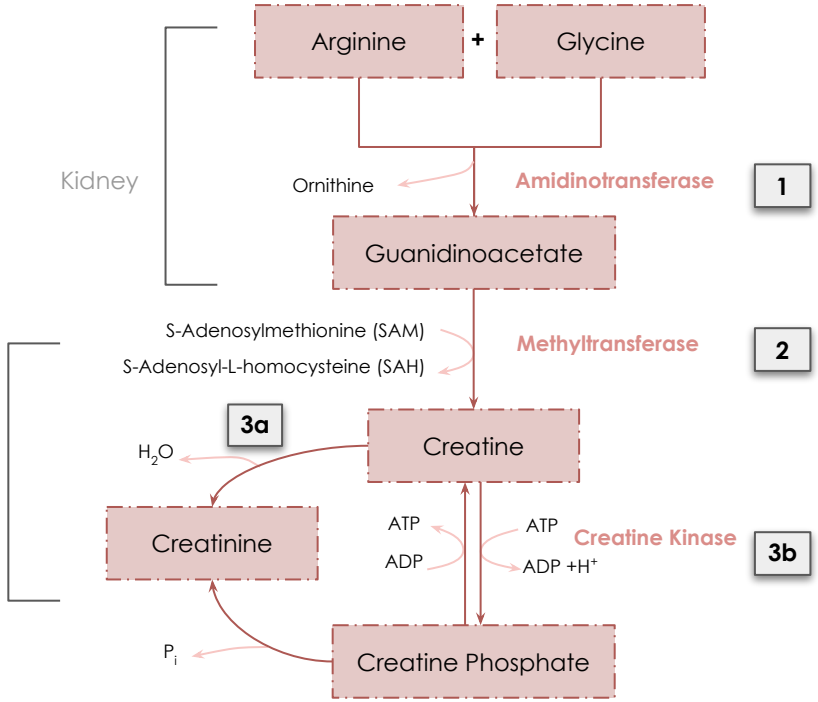
Now we have Two possibilities:

3a Creatine ⟶ Creatinine

- H_2O Out.
- Creatine in liver will remove water and become creatinine in kidney to get excreted (discussed in details in a bit)

3a Creatine ⇄ (**Creatine Kinase**) Creatine Phosphate

- ATP In.
- ADP Out.
- Moving a phosphate group from an ATP and adds it to Creatine to become Creatine Phosphate by the help of **Creatine Kinase** enzyme and be kept inside muscles. (discussed in details in a bit)



★ extra explanation

To make it very clear:

- Imagine Arginine as pasta (main substrate)
- Glycine as pasta sauce (we need glycine for this reaction as much as we need sauce for pasta)
- Amidotransferase is the container that will boil and cook the pasta
- Pasta is ready! (Guanidinoacetate)

Now listen, here the guanidinoacetate will be converted to Creatine by **methyltransferase**

You now have two possibilities:

- Either Creatine in liver will remove water and become creatinine in kidney to get excreted

OR

- Creatine will release a phosphate group from an ATP to become Creatine Phosphate and be kept inside muscles. Reason for this:
  - When muscle contracts the body takes time to start glycolysis so meanwhile Creatine phosphate will convert back to creatine
  - This will bring back the ATP consumed in the first place. A method of getting some energy since glycolysis will not start immediately (usually this process occurs within the first seconds of muscle contraction).
  - That's why bodybuilders take Creatine as a supplement, to enhance their kick off at exercises hence more efforts will be produced.

★ extra explanation

Summary of the Creatine Biosynthesis in order for you to gain a better understanding In tables

Reaction 1	
Reactant	Arginine + glycine
Product	Guanidinoacetate
Enzyme	**Amidinotransferase**
Action	Join the two amino acid
Byproduct	Ornithine

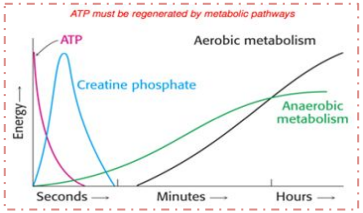
Reaction 2	
Reactant	Guanidinoacetate
Product	Creatine
Enzyme	**Methyltransferase**
Action	Add methyl group to the Guanidinoacetate
Consume	SAM

Reaction 3a	
Reactant	Creatine
Product	Creatinine
Enzyme	Remove water
Action	Creatine in liver will remove water and become creatinine in kidney to get excreted
Consume	-

Reaction 3b	
Reactant	Creatine
Product	Creatine Phosphate
Enzyme	**Creatine Kinase**
Action	Moving a phosphate group from an ATP and adds it to Creatine
Consume	ATP

Creatine Phosphate

- It's A high-energy phosphate compound Acts as a storage form of energy in the muscle.
- Provides small but, ready source of energy during first few seconds of intense muscular contraction (first source of ATP during the exercises).
- The amount of creatine phosphate in the body is proportional to the muscle mass (it's variable depending on the muscle mass More muscle → more creatine)

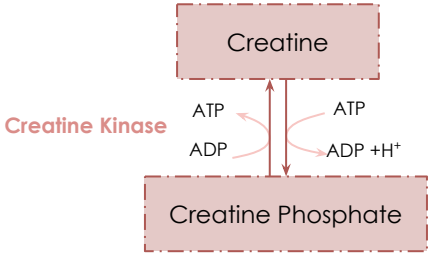


Creatine Degradation

- Creatine and Creatine phosphate spontaneously form creatinine (without the need for enzymes) as an end product which is excreted by the kidney in urine.
- Serum creatinine is a sensitive indicator of kidney disease "kidney function test" (it's excreted by glomerular filtration in the kidney) so it will increase with the impairment of kidney function. (a biomarker)
- Creatine and Creatine phosphate are non-enzymatic (they don't require any enzyme).
- In other meanings, when you take a 24 hours urine sample from a patient and find that creatinine levels were high, you will most probably think that the kidney is not functioning correctly (not necessarily a kidney failure tho)
- If you recall foundation block, Cystatin C is a kidney biomarker as well for assessing GFR and kidney function, it is better than creatinine because it doesn't depend in muscle mass, age, or gender.

Urinary Creatinine

- A typical male excretes about 15 mmol creatinine/day.(1gm-2gm)
- Recalling the steady state therefore the daily dose is 1-2 gm as well.
- Decrease in muscle mass (in muscular dystrophy, paralysis) leads to decreased level of urinary creatinine.
- The amount of creatinine in urine is used as an indicator for the proper collection of 24 hours urine sample.
- CK can be used in two reactions (reversible reactions):
    1. Generating creatine phosphate, by taking one phosphate group from ATP and convert it to ADP (during rest).
    2. Generating ATP from creatine phosphate, by taking the phosphate from it (C~P) and adds it to ADP (during intense and immediate muscle contraction).



Creatine Kinase (CK)

- CK is responsible for generation of energy (Creatine) in contractile muscular tissues.as it is the enzyme that used to regenerate ATP from creatine phosphate.
- CK levels change in cardiac and skeletal muscle disorders because it is mostly found in these tissues (can be used as a biomarker) but troponin is better than it for cardiac assessment.
- If there's a change in the CK it will indicate a muscle disorder.
- If the person have myocardial infarction we all know that the gold standard is measuring troponin but CK is also measured.
- Each tissue has a different form of creatine kinase (CK) such as (isozyme):
    - CK in Brain (CKBB)
    - CK in skeletal muscle (CKMM)
    - CK in Cardiac (CKMB)

Collagen

- Most abundant protein in the human body and it has a long rigid structure.(20-30% of total body proteins)
- Collagen is a highly stable molecule with a half-life as long as several years.
- A fibrous protein that serves structural functions.(sometimes with a bit of flexibility)
- Part of: connective tissues, bone, teeth, cartilage, tendons, skin and blood vessels.
- Collagen Type I: Skin , Tendons and Bones.
- Collagen Type II: Cartilage.
- Type III: Reticulate (reticular fibers).
- Type IV: Basement membrane.
- Type V: Cell surfaces, hair and placenta.

- The placenta is an organ that develops in the mother's uterus during pregnancy, it provides O_2 and nutrients to the fetus as well as removing the waste products from the baby's blood.
- Collagen fibers support body tissues, plus collagen is a major component of the extracellular matrix that supports cells. Collagen and keratin give the skin its strength, waterproofing, and elasticity. Loss of collagen is a cause of wrinkles. Collagen production declines with age, plus the protein can be damaged by smoking, sunlight, and other forms of oxidative stress.

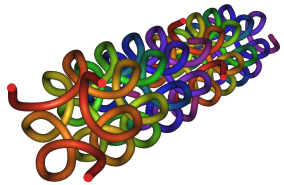
Collagen Structure

A helpful video

- Collagen a-chain (~1,000 amino acids long) is rich in proline and glycine.
- The glycine residues are part of a repeating sequence of: $(Gly - pro - Hyp)_{333}$

- Gly-X-y-

- **X** = Frequently Proline
- Y = Often hydroxyproline or hydroxylysine (The third amino acid in collagen can be any amino acid)
- Easy and simple structure of collagen clarifies its abundance in the body.
- Clinical correlation:
  - Osteogenesis imperfecta, a bone disease which results in brittle bones. Caused by defect in type I collagen due to a substitution of an amino acid within the collagen and interferes with the normal folding of collagen.
- **To warm it up:**
  1. It's a protein: Collagen is a polypeptide chain, made of 3 amino acids.
  2. Composition of the peptide chain: Glycine is always involved, proline is usually found. The third can differ or sometimes hydroxyproline.
  3. Observation of the chain:
     - 3 alpha chains wounded around each other. Forming a triple helix structure.
  4. Stability: They are held together by hydrogen bonds thus its stable

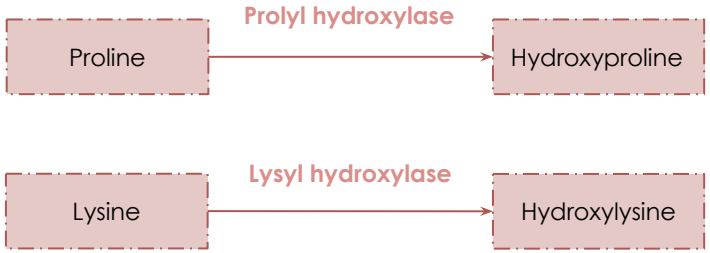


Collagen Structure, Contd...

- Collagen consists of three a-chains wound around one another in a rope-like triple helix (not an a-helix)
- The three polypeptide chains are held together by hydrogen bonds (interchain hydrogen bonds). interchain= hydrogen bond between the chains while intrachain= within the chains
- Two examples of protein secondary structure: collagen helix and a-helix
- Rich in proline and glycine amino acids
- Proline prevents collagen chains to form a-helix because:
  - Proline has no backbone amino group (it is a ring structure with secondary amino group). Therefore hydrogen bonding within the helix is not possible "Because it has so much proline so it will prevent the hydrogen bonds within the helix".
- Collagen helix is the triple helix made of 3 wounded alpha helices.

Non-standard Amino Acids in Collagen

- Non-standard Amino Acids are amino acids that are not encoded within the genome but can be made within the cell after the amino acid's synthesis, when needed, they are made from the modification of non-essential amino acids.
- During post-translational modifications proline and lysine converted to:



- The enzyme requires vitamin C for its function.
- If vitamin C is decreased, the amount of the enzyme (hydroxylase) will be deficient and as a result (Proline and Lysine) won't be hydroxylated and the resulting collagen will be defected (doesn't have proper strength)

Types of collagen

- Types of collagen depend on function and location.
- Variations in the amino acid sequence of a-chains result in different properties.
- Examples:
  1. $a_1\ a_1\ a_2$
  2. $a_1\ a_1\ a_1$

Fibril forming (Type 1,2 and 3) Used for more strength	Type I	Type II	Type II
	Skin, bone, tendon, blood vessels and cornea	Cartilage, intervertebral disk and vitreous body	Blood vessels, skin and muscle
Network forming (Type 4 and 7)	Type IV	Type VII	
	Basement membrane	Corneal and vascular endothelium	
Fibril associated (Type 9 and 12) Used to link fibers to each others or to other molecules	Type IX	Type XII	
	Cartilage	Tendon, ligaments , some other tissues	

original pic

Biosynthesis of collagen

- Synthesized in fibroblasts, osteoblasts and chondroblasts.
- Pre-Pro → Pro → tropocollagen → Mature collagen.
- Polypeptide precursors are enzymatically modified to form triple helix.
- Hydroxylation of proline and lysine residues.
- Glycosylation of some hydroxylysine residues with glucose or galactose.
- Secreted from Golgi vacuoles into the extracellular matrix as procollagen.
- Cleaved by N- and C- procollagen peptidases to release triple helical tropocollagen molecules.

1 **In ER:** Genes for Pro-a_1 and Pro-$a_2$chains are transcribed into mRNA.

2 **In ER:** mRNA is translated on the RER into prepro-a polypeptide chains that are extruded into the lumen of the RER, where the signal sequence is removed.

3 **In ER:** Hydroxylation of selected proline and lysine residues by **Prolyl and Lysyl hydroxylase** enzyme.

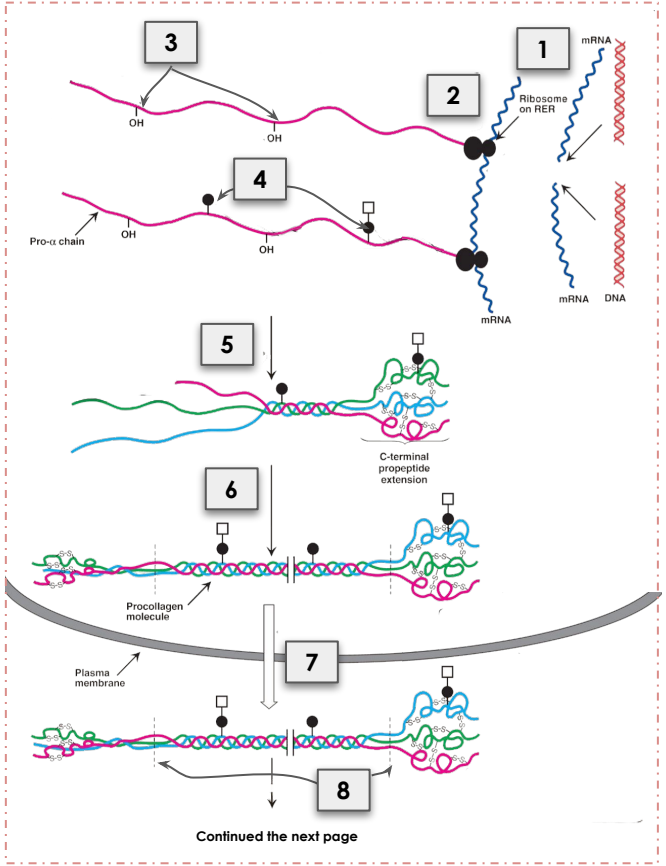
4 **In Golgi Apparatus:** Glycosylation of some selected hydroxylysine residues with glucose or galactose.

5 **In Golgi Apparatus:** Three pro-a chains assemble, intrachain "within the chain" and interchain "between the chains" disulfide bonds form at the C-terminal propeptide extension.

6 **In Golgi Apparatus:** A triple helix is formed and procollagen is produced.

7 **In Golgi Apparatus:** The procollagen molecule is secreted from Golgi vacuoles into the extracellular matrix a

8 **In ECF** "matrix"**:** N-terminal and C-terminal propeptides are cleaved by **N- and C-procollagen peptidases** to release triple helical tropocollagen molecules



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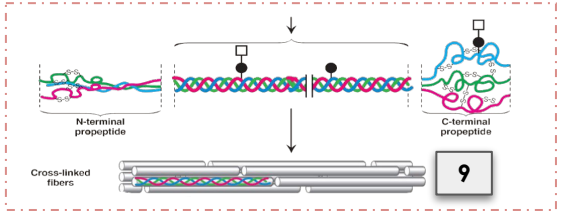
Biosynthesis of collagen, Contd...

9 **In ECF** "matrix"**:** Self-assembly of tropocollagen molecules into collagen fibrils, with subsequent cross-linking to form mature collagen fibers.



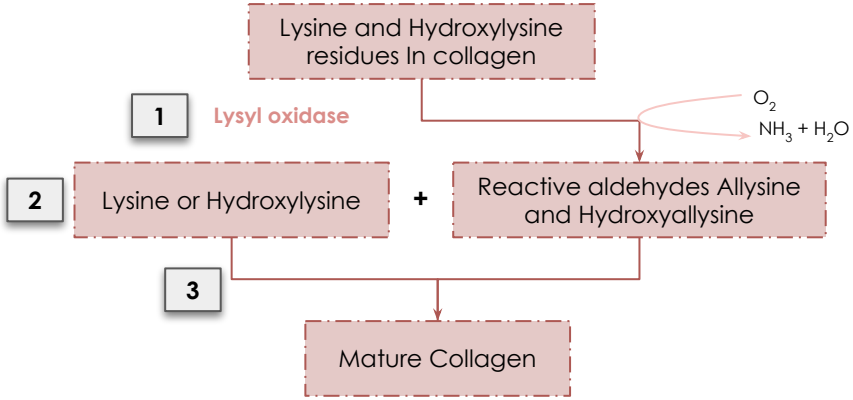
Final Step and Crosslinking

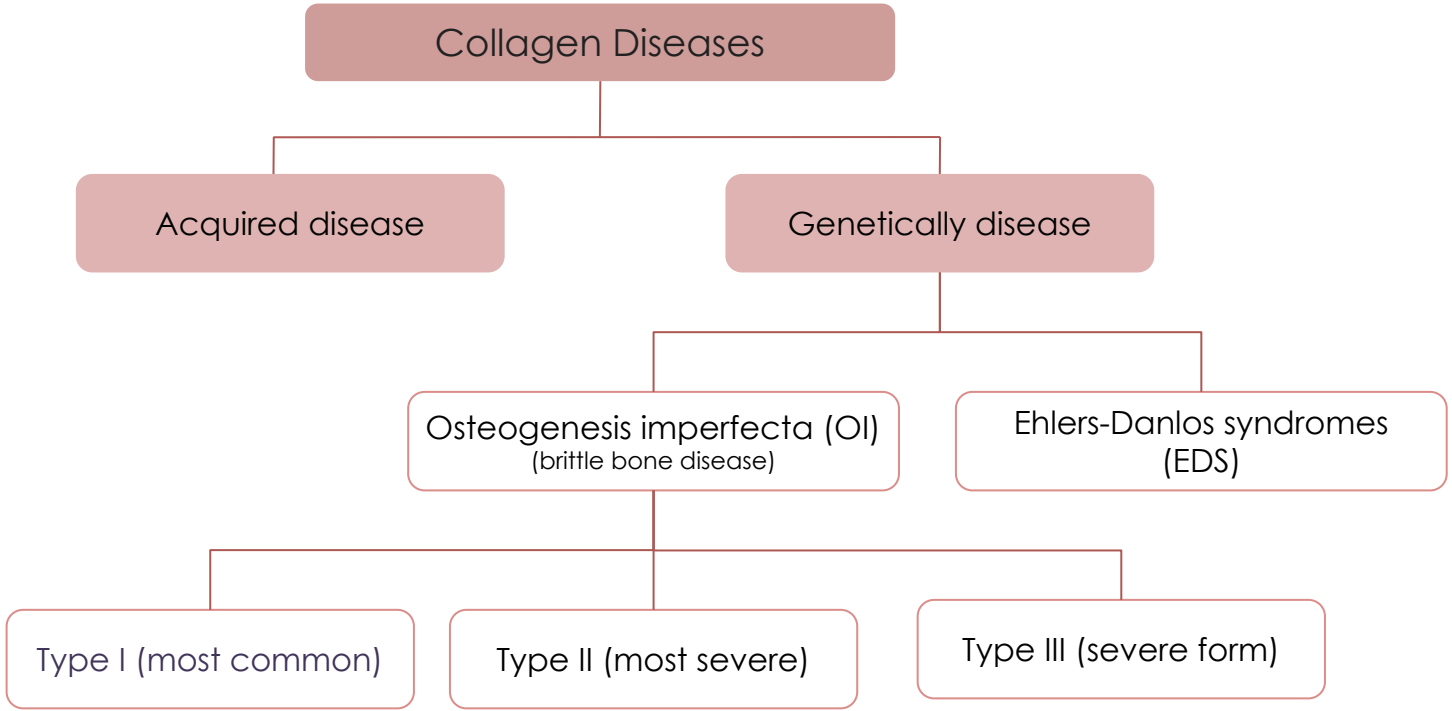
1 **Lysyl oxidase** oxidatively deaminates some of the lysine and hydroxylysine residues in collagen."In tropocollagen we have lysine and hydroxylysine"
(By removing the amino group from lysine and hydroxylysine residues).

- O_2 In.
- $NH_3 + H_2O$ Out.

2 The reactive aldehydes – Allysine and Hydroxyallysine condense with lysine or hydroxylysine residues in neighboring collagen molecules to form covalent cross-links, the reactive aldehyde (allysine and hydroxyaallysine) will make bonds with lysine and hydroxylysine (Deaminated collagen chain binds with non-deaminated collagen chain)

3 This produces mature collagen fibres.




Collagen Diseases
Acquired disease
Genetically disease
Osteogenesis imperfecta (OI)
(brittle bone disease)
Ehlers-Danlos syndromes
(EDS)
Type I (most common)
Type II (most severe)
Type III (severe form)

Collagen Diseases

- **Acquired disease** (also called sailors disease):
- Scurvy due to vitamin C deficiency.
- This disease is due malnutrition and it mainly affects (gums and teeth).
- Symptoms:
  - ↳ Bleeding gums, the gums become spongy, painful and inflamed.
  - ↳ Detaching of the teeth from gums.
  - ↳ Skin problems
  - ↳ Enzyme affected: hydroxylase.

- **Genetically disease:**

1. Ehlers-Danlos syndromes (EDS):
- Due to deficiency of lysyl hydroxylase or N-procollagen peptidase.
- Mutations in the amino acid sequences of collagen I, III and V.
- Characterized by hyperextensibility of joints and skin.

2. Osteogenesis imperfecta (OI) "brittle bone disease"
- Bones fracture easily with minor or no trauma.
- Mutations replace glycine with amino acids having bulky side chains preventing the formation of triple helical conformation.
  - ↳ Type I (most common): characterized by mild bone fragility, hearing loss and blue sclerae.
  - ↳ Type II (most severe): and lethal in the prenatal period (fractures in utero), Click here for a related clinical case of OI type II
  - ↳ Type III (severe form): Fractures at birth, short stature and spinal curvature leading to a humped back (kyphotic) appearance and blue sclerae.

Quiz

Q1: Disease cause by Mutations replace glycine with amino acids having bulky side chains?			
A) Acquired disease	B) Ehlers-Danlos syndromes (EDS)	C) Osteogenesis imperfecta (OI)	D) Pompe disease
Q2 : Hydroxylation of proline and lysine residues by which enzyme ?			
A) N-procollagen peptidase	B) L-oxidase	C) hydroxylase	D) Methyltransferase
Q3 : 98% of body creatine is in?			
A) Liver	B) Heart	C) Kidney	D) Muscle
Q4 : Methyltransferase will convert guanidinoacetate to …….. ?			
A) Creatine	B) Creatine phosphate	C) Ornithine	D) Glycine
Q5 : Scurvy (Acquired disease) caused due to?			
A) Deficiency N-procollagen peptidase	B) Deficiency of Lysyl hydroxylase	C) Mutations in the amino acid sequences	D) Deficiency of vitamin C

SAQs :

Q1: What are the three amino acids required for creatine biosynthesis?

Q2: Why does proline prevents collagen chains o form a-helix?

★ **MCQs Answer key:**

1) C 2) C 3) D 4) A 5) D

★ **SAQs Answer key:**

1) 1- Glycine 2- Arginine 3- Methionine

2) Slide 11

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"Your positive action combined with positive thinking results in success".

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