

Free Radicals and Anti-Oxidants

? FREE RADICALS

? Definition

A **free radical** is any atom or molecule with **one or more unpaired electrons** in its outer orbit, making it highly reactive.

? Examples

- Superoxide ($\text{O}_2^{\bullet-}$)
- Hydroxyl radical ($\bullet\text{OH}$)
- Nitric oxide ($\text{NO}^{\bullet}$)
- Peroxyl radicals ($\text{ROO}^{\bullet}$)

? Sources

- Mitochondrial ETC leakage
- Cytochrome P450 reactions
- Inflammation (neutrophils/macrophages)
- Radiation (UV, X-ray)

- Pollution, cigarette smoke
- Drugs and toxins

? REACTIVE OXYGEN SPECIES (ROS)

? Definition

Highly reactive oxygen-containing molecules, including radicals and non-radicals.

? Major ROS

Free radicals:

- Superoxide ($O_2^{\bullet-}$)
- Hydroxyl radical ($\bullet OH$)
- Peroxyl radicals ($ROO\bullet$)

Non-radicals that generate radicals:

- Hydrogen peroxide (H_2O_2)
- Hypochlorous acid ($HOCl$)
- Ozone (O_3)

? GENERATION OF FREE RADICALS & ROS

? Physiological pathways

- **Mitochondrial ETC leakage** ? 1–3% of O_2 becomes superoxide.
 - **Phagocytosis respiratory burst** (NADPH oxidase):
 $O_2 \rightarrow O_2^{\bullet -} \rightarrow H_2O_2 \rightarrow HOCl$
(important for neutrophil killing)
 - **Cytochrome P450** reactions
 - **Prostaglandin/catecholamine metabolism**
 - **Xanthine oxidase** during ischemia–reperfusion injury.
-

? Pathological generation

- Ionizing radiation (splits water ? **$\bullet OH$ radicals**)
 - UV light
 - Smoking, air pollution
 - Ischemia–reperfusion injury
 - Chronic inflammation
 - Heavy metals (Fe^{2+} via Fenton reaction)
-

? CELLULAR DAMAGE BY FREE RADICALS

ROS cause **oxidative stress**, damaging:

? 1. Lipids ? Lipid Peroxidation

ROS attack **polyunsaturated fatty acids** in membranes.

Effects:

- Loss of membrane integrity
- Increased permeability
- Cell swelling
- Mitochondrial damage

Highly exam-relevant for:

- Liver injury
- Atherosclerosis
- Radiation toxicity

? 2. Proteins ? Protein Oxidation

ROS cause:

- Cross-linking
- Fragmentation
- Enzyme inactivation

Seen in:

- Aging
 - Cataract formation
 - Neurodegenerative diseases
-

? 3. DNA ? DNA Damage

•OH causes:

- Base modification
- Strand breaks
- Mutations ? cancer risk

Seen in:

- UV radiation exposure
 - Smoking
 - Radiation therapy
-

? FREE RADICAL SCAVENGER SYSTEMS (ANTIOXIDANT DEFENSES)

Antioxidant mechanisms protect cells from ROS.

? A. ENZYMATIC ANTIOXIDANTS (VERY HIGH YIELD)

? 1. Superoxide Dismutase (SOD)

Converts:

$O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2$

Types:

- Cytosolic (Cu/Zn-SOD)
- Mitochondrial (Mn-SOD)

? 2. Catalase

Present in **peroxisomes**.

Converts:

$2H_2O_2 \rightarrow 2H_2O + O_2$

? 3. Glutathione Peroxidase (GPx)

Contains **selenium**.

Reduces:

$H_2O_2 \rightarrow H_2O$

Lipid peroxides $\rightarrow$ alcohol

Uses reduced glutathione (GSH).

? 4. Glutathione Reductase

Regenerates GSH using **NADPH**.

? B. NON-ENZYMATIC ANTIOXIDANTS

? 1. Vitamin E (?-tocopherol)

- Most important lipid-phase antioxidant
 - Prevents lipid peroxidation in membranes
-

? 2. Vitamin C (ascorbate)

- Regenerates vitamin E
 - Scavenges hydroxyl radicals
 - Important in smokers and chronic inflammation
-

? 3. Vitamin A & Carotenoids

- Quench singlet oxygen
 - Protective in UV light exposure
-

? 4. Glutathione (GSH)

- Removes peroxides

- Maintains redox balance
 - Reduced form necessary for GPx activity
-

? 5. Uric Acid

- Scavenges singlet oxygen & hydroxyl radicals
-

? 6. Bilirubin

- Powerful chain-breaking antioxidant in plasma
 - Elevated in newborns as protective mechanism
-

? 7. Transferrin, Ferritin

Bind Fe^{2+} , prevent Fenton reaction.

? BODY SYSTEMS PROTECTING AGAINST ROS

- **NADPH**: Needed for glutathione regeneration
 - **Thioredoxin system**
 - **Ceruloplasmin**: Neutralizes free copper
 - **Metallothioneins**: Bind heavy metals
-

- **Albumin:** Scavenges free radicals in plasma

? CLINICAL RELEVANCE

? High ROS found in:

- Atherosclerosis
- Diabetes mellitus
- Alcoholic liver disease
- Parkinson's & Alzheimer's diseases
- COPD
- Ischemia–reperfusion injury
- Cancer initiation (DNA damage)

? Antioxidant therapies:

- Vitamin E & C
- N-acetylcysteine (NAC)
- Selenium sources
- Polyphenols

? ULTRA-SHORT EXAM NOTES

- Free radicals have **unpaired electrons** ? high reactivity.
- ROS include **$O_2^{\bullet-}$, H_2O_2 , $\bullet OH$, $ROO\bullet$, $HOCl$** .
- Major sources: **ETC leakage, inflammation, radiation, P450, reperfusion**.
- Lipid peroxidation ? membrane damage (most common).
- Key enzymes: **SOD, catalase, GPx**.
- GSH + NADPH are essential for antioxidant defense.
- Vitamin E = membrane protector; Vitamin C = aqueous protector.

? INFLAMMATION & FREE RADICALS

? How ROS Are Generated in Inflammation

During acute and chronic inflammation, **neutrophils and macrophages** produce large amounts of ROS through:

1. Respiratory Burst (NADPH Oxidase System)

$O_2 \rightarrow O_2^{\bullet-} \rightarrow H_2O_2 \rightarrow HOCl$

- Superoxide produced by **NADPH oxidase**
- H_2O_2 generated by **superoxide dismutase**
- $HOCl$ generated by **myeloperoxidase (MPO)**

2. Nitric Oxide ($NO\bullet$) Production

- Macrophages produce **NO**
 - NO reacts with O_2 to form **Peroxynitrite (ONOO $^-$)**, a strong oxidant
-

? Effects of ROS in Inflammation

- Damage to invading microbes
 - Also injures host tissues → **oxidative stress**
 - Vascular endothelial damage
 - Increased permeability & edema
 - Promotion of chronic inflammation (arthritis, IBD)
-

? RESPIRATORY DISEASES & ROS

Free radicals contribute to major lung diseases.

? Chronic Obstructive Pulmonary Disease (COPD)

Sources of ROS:

- Cigarette smoke (very rich in free radicals)
 - Activated neutrophils in airways
 - Damaged mitochondria
-

Effects:

- Destruction of alveolar walls
 - Elastin fragmentation ? emphysema
 - Persistent inflammation
-

? Asthma

- Eosinophils produce ROS
 - Oxidative stress ? airway hyperresponsiveness
 - Decreases activity of **antioxidants (SOD, catalase)**
-

? Interstitial Lung Disease

- Persistent inflammation ? ROS-mediated fibrosis
 - Seen in pneumoconiosis, radiation lung injury
-

? Acute Respiratory Distress Syndrome (ARDS)

- Neutrophil burst ? HOCl, O₂•?
 - Endothelial damage ? capillary leakage
-

- Surfactant destruction ? respiratory failure

? Overall

Oxidative stress is one of the **central mechanisms** of chronic and acute respiratory diseases.

? RETROLENTAL FIBROPLASIA (RETINOPATHY OF PREMATURITY)

A classic exam favourite.

? Cause

Occurs in **premature infants** exposed to **high oxygen therapy**.

? Mechanism (Oxidative Pathway)

1. **High O₂** ? retinal vessels constrict (vaso-occlusion).
2. Withdrawal of oxygen ? retina becomes relatively **hypoxic**.
3. Hypoxia triggers **VEGF** release ? excessive neovascularization.
4. Fragile new vessels bleed ?
 - Fibrous scarring
 - Retinal detachment
 - Blindness

? Free Radical Link

High oxygen environment increases ROS (especially in immature retina lacking antioxidant enzymes), contributing to capillary damage.

? Prevention

- Controlled oxygen therapy
- Pulse oximetry targeting **90–95% saturation**
- Screening of preterm infants

? REPERFUSION INJURY

? Definition

Tissue damage that occurs **after blood supply returns** to previously ischemic tissue.

? Why it happens

Reintroduction of oxygen leads to sudden burst of ROS:

? Major ROS Sources

1. **Mitochondrial respiration restart**
2. **Xanthine oxidase** converts accumulated hypoxanthine ? uric acid, producing $O_2^{\bullet-}$
3. **Activated neutrophils** produce ROS

4. Cytochrome P450 leakage

? Consequences

- Lipid peroxidation of membranes
 - Mitochondrial permeability transition ? cell death
 - Calcium overload
 - Endothelial swelling & thrombosis
 - Worsened infarct size
-

? Clinical Conditions with Reperfusion Injury

- **Myocardial infarction** after thrombolysis
 - **Stroke** after revascularization
 - **Organ transplantation**
 - **Tourniquet release in trauma**
 - **Mesenteric ischemia**
-

? Antioxidant Protection

- SOD, catalase, GPx
- Vitamin C/E
- Allopurinol (prevents xanthine oxidase-mediated ROS)
- N-acetylcysteine

? ULTRA-SHORT REVISION BOX

- Inflammation generates ROS via **NADPH oxidase & MPO**.
- Respiratory diseases involve excessive oxidative stress from smoking & inflammation.
- Retrolental fibroplasia: **hyperoxia ? vasoconstriction ? hypoxia ? VEGF ? blindness**.
- Reperfusion injury: sudden **ROS overload** after restoring blood supply.

? ATHEROSCLEROSIS & FREE RADICALS

Free radicals and oxidative stress play a central role in the initiation and progression of atherosclerosis.

? How ROS contribute to atherosclerosis

1. Oxidation of LDL (Major Mechanism)

- ROS convert **LDL ? oxidized LDL (oxLDL)**.

- oxLDL is taken up by macrophages ? **foam cells**.
- Foam cells form **fatty streaks**, the earliest atherosclerotic lesions.

2. Endothelial Damage

- ROS injure endothelial cells ? reduced nitric oxide ? vasoconstriction.
- Damaged endothelium becomes **adhesive**, promoting leukocyte entry.

3. Smooth Muscle Proliferation

- ROS stimulate smooth muscle migration into intima ? plaque growth.

4. Inflammation Enhancement

- ROS activate NF- κ B and inflammatory cytokines ? chronic vascular inflammation.

? Clinical relevance

- Hyperlipidemia + ROS = accelerated plaque formation
- Smokers have extremely high oxidative LDL burden
- Antioxidants (Vitamin E, polyphenols) may reduce progression
- Diabetes increases ROS ? enhances atherosclerotic risk

? SKIN DISEASES & FREE RADICALS

Many skin pathologies are driven or worsened by oxidative stress.

? 1. Photoaging (UV-induced skin aging)

- UV light ? generates ROS in skin
- ROS damage collagen, elastin ? **wrinkles, laxity**
- Increases matrix metalloproteinases ? collagen breakdown

? 2. Melasma & Hyperpigmentation

- ROS stimulate melanocytes
- Increase melanin production ? dark patches
- Antioxidants used in cosmetic dermatology (vitamin C, niacinamide)

? 3. Acne

- Neutrophils in comedones generate ROS
- Increase inflammation & tissue damage
- Some anti-acne formulations include antioxidants

? 4. Psoriasis

- Hyperproliferation of keratinocytes produces ROS

- Oxidative stress amplifies inflammation

? 5. Vitiligo

- Excess hydrogen peroxide in skin damages melanocytes
- Antioxidants (calcium folinate, vitamin C/E, topical catalase mimetics) support treatment

? 6. Atopic Dermatitis

- Chronic inflammation ? ROS generation
- Barrier disruption increases oxidative injury

? AGE-RELATED DISEASES & FREE RADICALS

Aging is strongly connected with cumulative oxidative damage — the basis of the **Free Radical Theory of Aging**.

? 1. Neurodegenerative Disorders

? Alzheimer's Disease

- ?-amyloid generates ROS
- Lipid peroxidation + protein carbonylation
- Mitochondrial dysfunction

? Parkinson's Disease

- Dopamine oxidation ? ROS
 - Mitochondrial Complex I defect ? more ROS
 - Damages substantia nigra neurons
-

? 2. Cataract

- Lens proteins oxidize ? clouding
 - UV exposure accelerates oxidation
 - Reduced glutathione (GSH) in older people worsens progression
-

? 3. Aging Skin

- UV ? ROS ? collagen degradation
 - Elastin damage ? wrinkles
 - Reduced antioxidant enzymes with age
-

? 4. Sarcopenia (Age-related muscle loss)

- Mitochondrial ROS damage muscle fibers

- Decreased ATP ? reduced muscle strength
 - Impaired repair mechanisms
-

? 5. Cardiovascular Aging

- Endothelial nitric oxide decreases due to ROS
 - Increased arterial stiffness
 - Higher risk of hypertension, thrombosis, and MI
-

? 6. Immune Senescence

- ROS damage lymphocytes and stem cells
 - Reduced adaptive immunity
 - Increased infections and cancer susceptibility
-

? 7. Diabetes & Aging

- Hyperglycemia ? advanced glycation ? ROS burst
 - Mitochondrial oxidative stress ? insulin resistance
 - Vascular complications (retinopathy, nephropathy)
-

? ULTRA-SHORT REVISION

- **Atherosclerosis:** Oxidized LDL ? foam cells ? plaques.
- **Skin diseases:** ROS central in UV-damage, melasma, vitiligo, psoriasis, acne.
- **Age-related diseases:** Alzheimer's, Parkinson's, cataract, skin aging, sarcopenia linked to cumulative ROS injury.
- Antioxidant enzymes (SOD, catalase, GPx) weaken with age ? more oxidative damage.

? LIPID PEROXIDATION

? Definition

Oxidative degradation of **polyunsaturated fatty acids (PUFAs)** in cell membranes by free radicals.

? Why it matters

- Major mechanism of **cell membrane damage**
- Leads to loss of membrane fluidity, integrity & function
- Produces toxic aldehydes (e.g., **malondialdehyde – MDA**), a marker of oxidative stress

? Where it occurs

- Biological membranes rich in PUFAs (liver, brain, skin)
- Lipoproteins (oxidized LDL ? atherosclerosis)

? MECHANISM OF LIPID PEROXIDATION

Lipid peroxidation follows **three classic phases**:

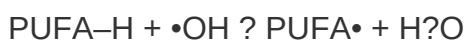
? 1. Initiation Phase

ROS + PUFA ? **Lipid radicals** ($L\cdot$)

Key initiators:

- **Hydroxyl radical** ($\cdot OH$) — most potent
- Peroxynitrite ($ONOO\cdot$)
- UV radiation
- Ionizing radiation
- Heavy metals (Fe^{2+} via Fenton reaction)

Reaction:



(The $\cdot OH$ removes H ? creates lipid radical)

? 2. Propagation Phase

Lipid radical reacts with oxygen ? **lipid peroxy radical** ($LOO\cdot$)

LOO• reacts with nearby PUFA ? new **lipid radical (L•)**

This forms a **chain reaction**.

Cycle:

$L\bullet + O_2 \rightarrow LOO\bullet$

$LOO\bullet + PUFA-H \rightarrow LOOH + L\bullet$

Products:

- Lipid hydroperoxide (**LOOH**)
- Malondialdehyde (**MDA**)
- 4-HNE (4-hydroxynonenal)

These molecules damage:

- Membrane proteins
- DNA
- Mitochondria
- Lipoproteins

? 3. Termination Phase

Occurs when two radicals combine ? **non-radical** stable product.

$LOO\bullet + LOO\bullet \rightarrow$ non-radical

$L\bullet + LOO\bullet \rightarrow$ stable complex

OR

Stopped by **chain-breaking antioxidants** (vitamin E, vitamin C).

? PREVENTIVE ANTIOXIDANTS (HIGH-YIELD)

These **prevent formation of free radicals** *before* they start damaging lipids.

? Examples

? 1. Metal chelators

Prevent Fenton reaction ($\text{Fe}^{2+} \rightarrow \bullet\text{OH}$).

- **Transferrin**
- **Lactoferrin**
- **Ferritin**
- **Ceruloplasmin**
- **EDTA (lab)**

? 2. Enzymatic antioxidants

- **Superoxide dismutase (SOD)** ? removes $\text{O}_2^{\bullet-}$
- **Catalase** ? removes H_2O_2
- **Glutathione peroxidase** ? removes peroxides

? 3. Uric acid

Scavenges singlet oxygen.

? 4. Bilirubin

Protective chain-stopping antioxidant in plasma.

? 5. Albumin

Binds free copper/iron ? prevents radical formation.

Purpose: Stop ROS formation upstream, preventing initiation stage.

? CHAIN-BREAKING ANTIOXIDANTS

? Role

Interrupt the **propagation phase** by reacting with lipid peroxyl radicals (LOO•) and converting them into non-radical products.

? Key Chain-Breaking Antioxidants

? 1. Vitamin E (?-Tocopherol) — MOST IMPORTANT

- Lipid-soluble
- Present in membranes
- Neutralizes LOO• ? stops chain reaction
- First line of defense against lipid peroxidation

? 2. Vitamin C (Ascorbate)

- Water-soluble
- Regenerates **vitamin E**
- Scavenges •OH

? 3. Glutathione (GSH)

- Removes LOOH via glutathione peroxidase
- Restored by glutathione reductase + NADPH

? 4. Coenzyme Q (Ubiquinol)

- Membrane antioxidant
- Neutralizes lipid radicals

? 5. Carotenoids (?-carotene, lycopene)

- Quench singlet oxygen
- Protect lipids in membranes and skin

? 6. Polyphenols (tea, fruits)

- Donate hydrogen to radicals
- Stop propagation

? CLINICAL CONNECTIONS

? 1. Atherosclerosis

- LDL oxidation via lipid peroxidation ? foam cells ? plaque formation.

? 2. Skin Aging

- UV-induced lipid peroxidation damages collagen/elastin.

? 3. Neurodegeneration

- Brain is PUFA-rich ? vulnerable to ROS.

? 4. Liver damage

- Alcohol increases CYP2E1 ? ROS ? lipid peroxidation.

? 5. Cancer

- Lipid peroxidation byproducts like MDA are mutagenic.

? ULTRA-SHORT REVISION

- **Initiation:** ROS attacks PUFA ? lipid radical.
- **Propagation:** Lipid radical + O₂ ? peroxy radical ? chain reaction.
- **Termination:** Radicals combine OR antioxidants stop chain.
- **Preventive antioxidants:** Stop free radicals from forming.
- **Chain-breaking antioxidants:** Stop ongoing lipid peroxidation.

? IMPORTANT POINTS TO REMEMBER

? Lipid Peroxidation

- Occurs mainly in **polyunsaturated fatty acids (PUFAs)** of membranes.
- One of the most important mechanisms of **cell membrane injury**.
- Produces toxic byproducts like **malondialdehyde (MDA)** and **4-HNE**, used as markers of oxidative stress.
- Particularly important in **liver, brain, skin**, and **LDL particles**.

? Initiation Phase

- Triggered by **hydroxyl radical ($\bullet\text{OH}$)** ? most reactive species.
- Other initiators: UV, ionizing radiation, Fe^{2+} (Fenton reaction), peroxynitrite.
- ROS abstracts hydrogen from PUFA ? **lipid radical ($\text{L}\bullet$)**.

? Propagation Phase

- $\text{L}\bullet + \text{O}_2 \rightarrow$ **lipid peroxy radical ($\text{LOO}\bullet$)**.
- $\text{LOO}\bullet$ reacts with nearby PUFA ? **$\text{LOOH} + \text{new L}\bullet$** ? chain reaction.
- Responsible for rapid spread of membrane damage.
- Generates **lipid hydroperoxides (LOOH)** and **MDA**.

? Termination Phase

- Occurs when **two radicals combine** ? stable product.

- Chain-breaking antioxidants (e.g., vitamin E) stop radical propagation.
- Marks the end of lipid peroxidation.

? Preventive Antioxidants (Stop ROS Formation)

? Key Concept: Prevent the *initiation* phase.

- **SOD** ? removes superoxide ($O_2^{\bullet-}$).
- **Catalase** ? removes H_2O_2 .
- **Glutathione Peroxidase (selenium-dependent)** ? removes peroxides (H_2O_2 , LOOH).
- **Metal-chelating proteins**: transferrin, lactoferrin, ferritin, ceruloplasmin ? prevent Fenton reaction.
- **Albumin** ? binds free metals, prevents radical formation.
- **Uric acid** and **bilirubin** ? neutralize singlet oxygen.

? Chain-Breaking Antioxidants (Stop Propagation Stage)

? Key Concept: Break the radical chain by interacting with $LOO^{\bullet}$.

- **Vitamin E (α -tocopherol)** ? most important lipid-phase antioxidant; protects membranes.
- **Vitamin C (ascorbate)** ? regenerates vitamin E; scavenges aqueous radicals.
- **Glutathione (GSH)** ? detoxifies LOOH through GPx.
- **Coenzyme Q (ubiquinol)** ? membrane antioxidant.

- **Carotenoids** (?-carotene, lycopene) ? quench singlet oxygen.
- **Plant polyphenols** ? donate electrons to stop radical chains.

? High-Yield Clinical Pearls

- Oxidized LDL from lipid peroxidation ? **atherosclerosis**.
- UV-induced lipid peroxidation ? **photoaging**, melasma, skin inflammation.
- Alcohol metabolism (CYP2E1) increases ROS ? **fatty liver** and **hepatotoxicity**.
- High ROS in brain ? linked to **Alzheimer's and Parkinson's**.
- Newborn antioxidant systems are immature ? prone to **retinopathy of prematurity**.
- Reperfusion after ischemia produces ROS ? **further tissue injury**.

? Ultra-Short 10-Second Revision

- Lipid peroxidation = PUFA destruction by ROS.
- Steps: **Initiation ? Propagation ? Termination**.
- Preventive antioxidants stop radicals from forming.
- Chain-breaking antioxidants stop chain reactions already underway.
- Vitamin E = main membrane protector; GPx = removes peroxides.

1. A free radical is defined as a molecule that contains:

- A. Excess electrons
- B. Paired electrons
- C. **Unpaired electrons**
- D. No electrons

Answer: C

2. Which of the following is the MOST reactive oxygen species?

- A. Superoxide
- B. Hydrogen peroxide
- C. Singlet oxygen
- D. **Hydroxyl radical ($\bullet\text{OH}$)**

Answer: D

3. Which enzyme produces superoxide during inflammation?

- A. Xanthine oxidase
- B. **NADPH oxidase**
- C. Catalase
- D. Nitric oxide synthase

Answer: B

4. Hydrogen peroxide is converted to water and oxygen by:

- A. GPx
- B. SOD
- C. **Catalase**
- D. MPO

Answer: C

5. Which of the following is a non-radical ROS that can generate free radicals?

- A. $O_2^{\bullet-}$
- B. $\bullet OH$
- C. $NO\bullet$
- D. H_2O_2

Answer: D

6. The Fenton reaction requires which metal?

- A. Sodium
- B. Potassium
- C. **Iron (Fe^{2+})**
- D. Zinc

Answer: C

7. The primary site for mitochondrial ROS generation is:

- A. Golgi apparatus
- B. ER
- C. **Electron Transport Chain (ETC)**
- D. Peroxisome

Answer: C

8. The toxic aldehyde formed during lipid peroxidation and measured clinically is:

- A. Acetaldehyde
- B. Formaldehyde
- C. **Malondialdehyde (MDA)**
- D. Uric acid

Answer: C

9. Which phase of lipid peroxidation involves chain reaction amplification?

- A. Initiation
- B. **Propagation**
- C. Termination
- D. Stabilization

Answer: B

10. Which antioxidant is present in lipid membranes and stops propagation phase?

- A. Vitamin C
- B. Glutathione
- C. Uric acid
- D. **Vitamin E**

Answer: D

11. Which antioxidant regenerates Vitamin E?

- A. Coenzyme Q
- B. **Vitamin C**
- C. Bilirubin
- D. Selenium

Answer: B

12. Selenium is required for which enzyme?

- A. Catalase
- B. SOD
- C. **Glutathione peroxidase (GPx)**
- D. Xanthine oxidase

Answer: C

13. Preventive antioxidants primarily function by:

- A. Breaking lipid radicals
- B. Blocking LOO•
- C. **Preventing formation of new ROS**
- D. Stabilizing MDA

Answer: C

14. Which of the following is a preventive antioxidant?

- A. Vitamin E
- B. Vitamin C
- C. **Transferrin**
- D. Coenzyme Q

Answer: C

15. Lipid peroxidation is MOST harmful because it affects:

- A. DNA only
- B. Proteins only
- C. **Biological membranes**
- D. Cholesterol

Answer: C

16. Free radical injury is central to which ocular disease in preterm infants?

- A. Glaucoma
- B. Cataract
- C. **Retrolental fibroplasia (ROP)**
- D. Strabismus

Answer: C

17. Which free radical is produced during nitric oxide metabolism?

- A. $O_2^{\bullet}$
- B. $\bullet OH$
- C. **$NO^{\bullet}$**
- D. H_2O_2

Answer: C

18. In atherosclerosis, free radicals cause:

- A. LDL hydrolysis
- B. **LDL oxidation**
- C. LDL polymerization
- D. LDL glycation

Answer: B

19. Which enzyme converts $O_2^{\bullet}$ to H_2O_2 ?

- A. Catalase
- B. GPx
- C. **SOD**
- D. MPO

Answer: C

20. The final step (termination) of lipid peroxidation occurs by:

- A. Creating more radicals
- B. **Combining two radicals into a stable molecule**
- C. Breaking lipids into aldehydes
- D. Quenching hydroxyl radicals

Answer: B

21. Which ROS is mainly responsible for DNA strand breaks?

- A. $O^{\bullet}O^{\bullet}$
- B. $O^{\bullet}$
- C. $H^{\bullet}O^{\bullet}$
- D. $\bullet OH$

Answer: D

22. Which condition shows highest oxidative damage due to smoking?

- A. Hypothyroidism
- B. **COPD**
- C. Gallstone disease
- D. Asthma

Answer: B

23. Reperfusion injury occurs mainly due to:

- A. ATP accumulation
- B. Increased antioxidants
- C. **Sudden ROS burst after restoring oxygen**
- D. Excess nitric oxide removal

Answer: C

24. A major plasma antioxidant that binds copper and prevents radical formation is:

- A. Vitamin A
- B. **Ceruloplasmin**
- C. Myeloperoxidase
- D. Albumin

Answer: B

25. Which of the following is a chain-breaking antioxidant found in membranes?

- A. Catalase
- B. SOD
- C. **Vitamin E**
- D. Glutathione reductase

Answer: C

26. Which antioxidant is strongest in aqueous (cytosolic) phase?

- A. Vitamin E
- B. Coenzyme Q
- C. **Vitamin C**
- D. Bilirubin

Answer: C

27. Which condition is MOST strongly linked with H₂O₂ accumulation in skin?

- A. Melasma
- B. Acne
- C. **Vitiligo**
- D. Psoriasis

Answer: C

28. Which enzyme deficiency increases oxidative stress in RBCs?

- A. Lactate dehydrogenase
- B. Myeloperoxidase
- C. **G6PD deficiency**
- D. Catalase deficiency

Answer: C

29. Aging is accelerated by cumulative damage mainly to:

- A. Cholesterol
- B. Mitochondrial DNA**
- C. Lipoproteins
- D. Lysosomes

Answer: B

30. Which is the first-line antioxidant enzyme in mitochondria?

- A. Catalase
- B. GPx
- C. Mn-SOD**
- D. NADPH oxidase

Answer: C

? CLINICAL CASE-BASED QUESTIONS (Whole Chapter)

1. A 65-year-old smoker presents with progressive shortness of breath. CT chest shows emphysematous changes. Which mechanism contributes most to his lung damage?

- Cigarette smoke delivers **thousands of free radicals** per puff.
- ROS damage alveolar walls ? elastin breakdown ? emphysema.

Answer: Excess oxidative stress leading to lipid/protein oxidation in lungs.

2. A premature infant on high oxygen therapy develops retinal detachment and blindness. What is the underlying mechanism?

- Excess O₂ ? ROS overload ? capillary damage ? hypoxia on withdrawal ? VEGF-mediated neovascularization.

Answer: ROS-mediated vascular injury ? retrolental fibroplasia (ROP).

3. A 22-year-old G6PD-deficient male develops hemolysis after taking antimalarial drugs. Which mechanism is responsible?

- RBCs lack NADPH ? cannot regenerate **glutathione**.
- Peroxides accumulate ? membrane lipid peroxidation ? hemolysis.

Answer: Inability to detoxify ROS due to reduced glutathione.

4. A patient with MI is treated with thrombolysis. 30 minutes later, chest pain worsens and biomarkers rise again. Mechanism?

- Reperfusion ? sudden supply of oxygen ? ROS burst.
- Xanthine oxidase, neutrophils contribute to ROS.

Answer: Reperfusion injury due to sudden ROS generation.

5. A newborn has severe jaundice but mild oxidative stress. Which antioxidant explains this protection?

- Bilirubin acts as a natural antioxidant.

Answer: Elevated bilirubin functioning as an antioxidant.

6. A 48-year-old diabetic has decreased distal sensation. His labs show increased MDA levels. What process is elevated?

- MDA = marker of **lipid peroxidation**.

Answer: Increased lipid peroxidation due to chronic oxidative stress.

7. A patient presents with hyperpigmented patches worsened by sunlight. ROS involvement?

- UV generates ROS in skin ? stimulates melanocytes ? melasma.

Answer: UV-induced ROS activation of melanogenesis.

8. A 75-year-old man has memory loss, cerebral atrophy, and oxidative markers elevated. Underlying mechanism?

- Mitochondrial ROS damage neuronal lipids/proteins in Alzheimer's disease.

Answer: Oxidative stress-mediated neurodegeneration.

9. A smoker with coronary artery disease has high oxLDL levels. What caused this?

- ROS convert LDL ? oxidized LDL ? foam cells ? plaques.

Answer: Free radical-mediated oxidation of LDL in endothelium.

10. A 45-year-old alcoholic has fatty liver with high CYP2E1 activity. What does this promote?

- CYP2E1 generates ROS ? lipid peroxidation ? liver injury.

Answer: Alcohol-induced ROS formation causing hepatic lipid peroxidation.

11. A lab report shows high H₂O₂ accumulation in melanocytes in a vitiligo patient. Which enzyme defect is likely?

- Catalase deficiency ? H₂O₂ builds up ? melanocyte damage.

Answer: Reduced catalase activity causing H₂O₂ accumulation.

12. An ischemic limb becomes swollen and painful after tourniquet release. Why?

- Sudden oxygen reentry ? ROS burst ? inflammation, endothelial injury.

Answer: Reperfusion injury from ROS excess.

13. A 60-year-old man with chronic inflammation shows raised CRP and ROS markers. Neutrophils are responsible through which pathway?

- NADPH oxidase ? $O_2^{\bullet-}$? H_2O_2 ? HOCl (myeloperoxidase).

Answer: Respiratory burst generating ROS in inflammation.

14. A patient given excessive paracetamol develops liver failure. How does oxidative stress contribute?

- NAPQI depletes glutathione ? ROS accumulate ? hepatocyte necrosis.

Answer: GSH depletion ? inability to neutralize ROS.

15. A patient has premature wrinkles and skin aging. What is central mechanism?

- UV-induced ROS ? collagen & elastin destruction.

Answer: ROS-mediated photoaging of skin.

16. In COPD, what is the role of ROS from cigarette smoke?

- They destroy alveolar walls, activate neutrophils, damage surfactant.

Answer: Oxidative injury leading to emphysema development.

17. A 30-year-old athlete consumes high oxygen during intense exercise. Why do antioxidant enzymes rise?

- ETC leakage generates more ROS ? increased SOD, catalase, GPx.

Answer: Upregulation of enzymatic antioxidants due to increased ROS.

18. A term infant with respiratory distress is given high O₂; later lung fibrosis develops. Which ROS is responsible?

- Oxygen toxicity ? •OH formation ? alveolar damage.

Answer: Hydroxyl radical-mediated pulmonary injury.

19. Atherosclerotic plaque is examined and shows foam cells. What oxidized molecule is responsible?

- oxLDL due to lipid peroxidation.

Answer: Oxidized LDL formed by ROS.

20. A patient with Parkinson's disease shows increased oxidative stress in substantia nigra. What caused it?

- Dopamine metabolism produces ROS ? mitochondrial dysfunction.

Answer: ROS generation from dopamine oxidation.

? VIVA VOCE — Free Radicals & Antioxidants (Whole Chapter)

1. What is a free radical?

A molecule with **one or more unpaired electrons**, making it highly reactive.

2. Give examples of free radicals.

Superoxide ($O_2^{\bullet-}$), hydroxyl radical ($\bullet OH$), nitric oxide ($NO^{\bullet}$).

3. What is a reactive oxygen species (ROS)?

A reactive oxygen-containing molecule, radical or non-radical (e.g., H_2O_2 , $HOCl$).

4. Name the most dangerous ROS.

Hydroxyl radical ($\bullet OH$).

5. What is the primary source of ROS in the body?

Mitochondrial electron transport chain leakage.

6. What is respiratory burst?

Rapid ROS generation by neutrophils via **NADPH oxidase**.

7. Which enzyme converts superoxide into hydrogen peroxide?

Superoxide dismutase (SOD).

8. Which enzyme breaks down hydrogen peroxide?

Catalase or glutathione peroxidase (GPx).

9. Which antioxidant enzyme requires selenium?

Glutathione peroxidase.

10. What is the role of glutathione?

Neutralizes peroxides and maintains cellular redox balance.

11. What is lipid peroxidation?

ROS-mediated oxidation of **PUFAs** in membranes.

12. Name the three phases of lipid peroxidation.

Initiation, propagation, termination.

13. What starts the initiation phase?

Attack by **hydroxyl radical** on membrane lipids.

14. What is formed in propagation?

Lipid peroxyl radicals (LOO•) and **lipid hydroperoxides (LOOH)**.

15. How does the termination phase occur?

Two radicals combine to form a **stable non-radical**.

16. What is malondialdehyde (MDA)?

A toxic product of lipid peroxidation; marker of oxidative stress.

17. Give examples of preventive antioxidants.

SOD, catalase, GPx, transferrin, ferritin, ceruloplasmin.

18. Function of preventive antioxidants?

Prevent **formation** of ROS (act before initiation phase).

19. What are chain-breaking antioxidants?

Antioxidants that **stop propagation** by neutralizing $\text{LOO}\cdot$.

20. Example of a chain-breaking antioxidant.

Vitamin E (α -tocopherol).

21. How does Vitamin C act in antioxidant defense?

Regenerates **vitamin E** and scavenges aqueous radicals.

22. What antioxidant is abundant in plasma and binds copper?

Ceruloplasmin.

23. What is the physiological uncoupler in brown fat?

Thermogenin (UCP-1).

24. Which condition is strongly linked to LDL oxidation?

Atherosclerosis.

25. How does smoking increase oxidative stress?

Introduces large numbers of free radicals into lungs ? COPD.

26. How do ROS contribute to skin aging?

UV-generated ROS damage collagen and elastin.

27. Why is the brain highly susceptible to oxidative injury?

High PUFA content + high oxygen use + low antioxidants.

28. What causes reperfusion injury?

Sudden ROS burst when oxygen returns to ischemic tissue.

29. How do ROS participate in inflammation?

Neutrophils generate ROS (respiratory burst) for microbial killing.

30. What leads to retinopathy of prematurity?

Hyperoxia ? ROS ? retinal vessel damage ? neovascularization.

31. Which radical is responsible for DNA strand breaks?

Hydroxyl radical ($\bullet\text{OH}$).

32. How does glutathione peroxidase act on lipid peroxides?

Converts **LOOH** ? **alcohol**, preventing propagation.

33. Examples of non-enzymatic antioxidants.

Vitamin E, Vitamin C, glutathione, carotenoids, uric acid, bilirubin.

34. Which antioxidant is most important in membranes?

Vitamin E.

35. Why is catalase highly active in peroxisomes?

Peroxisomes generate large amounts of H_2O_2 .

36. What antioxidant protects newborns naturally?

Bilirubin.

37. Why does G6PD deficiency cause hemolysis?

Low NADPH ? low GSH ? peroxide accumulation in RBCs.

38. What is ONOO??

Peroxynitrite ? formed from $\text{NO}\bullet + \text{O}_2\bullet^-$? potent oxidant.

39. Which vitamins work together in antioxidant cycles?

Vitamin E and Vitamin C.

40. What is the main antioxidant in mitochondria?

Mn-SOD (manganese SOD).