NOTESKARTS | PREMIUM STUDY NOTES

BP812ET — DIETARY SUPPLEMENTS & NUTRACEUTICALS

UNIT — III | Free Radicals, ROS & Dietary Fibre as Functional Food

B.Pharm 8th Semester · PCI / AKTU Syllabus · Premium Notes ·

UNIT-III | LEARNING OBJECTIVES

After completing this unit, the student will be able to:

- LO-1: Define free radicals and Reactive Oxygen Species (ROS); classify them with examples, chemical formulae, and half-lives.
- LO-2: Explain the endogenous and exogenous sources of free radical production in cells, including the mitochondrial ETC, NADPH oxidase, Fenton reaction, and Haber-Weiss reaction.
- LO-3: Describe the mechanism and consequences of free radical-mediated damage to Lipids (lipid peroxidation chain reaction and products: MDA, 4-HNE, isoprostanes).
- LO-4: Explain oxidative damage to Proteins (carbonylation, nitration, cross-linking) and Carbohydrates (glycation, AGE formation, glycoxidation).
- LO-5: Describe free radical damage to Nucleic Acids — DNA base modifications, 8-OHdG as biomarker, strand breaks, and consequences (mutation, cancer, ageing).
- LO-6: Classify and explain Dietary Fibres and Complex Carbohydrates as functional food ingredients — their chemical nature, sources, physiological mechanisms, and health benefits.

INTRODUCTION TO FREE RADICALS

Definition and Characteristics

A **free radical** is any atom, molecule, or molecular fragment that has **one or more unpaired electrons** in its outer orbital. The unpaired electron makes it **highly reactive, short-lived, and capable of initiating chain reactions**. Free radicals are denoted by a dot (•) superscript, e.g., hydroxyl radical = **OH•**.

Free Radical	Any species capable of independent existence that contains one or more unpaired electrons. The unpaired electron creates paramagnetic properties and extreme chemical reactivity. (Halliwell & Gutteridge, 2007)

▸ Characteristics of Free Radicals

- Paramagnetic — attracted to magnetic fields due to unpaired electron spin.
- Extremely short half-life (nanoseconds to microseconds) — highly transient.

- Highly reactive — steal electrons from neighbouring molecules (oxidation).
- Self-propagating — chain reaction: one radical creates another.
- Both beneficial (at low levels: signalling, immunity) and harmful (at high levels: oxidative stress).

Notation, Electronic Structure & Classification

Type	Symbol	Nature	Half-life	Generation Site
Superoxide anion radical	$O_2^{\bullet -}$ ($O2^{\bullet -}$)	ROS — radical	Very short (microseconds)	Mitochondrial ETC (Complex I, III), NOX
Hydroxyl radical	OH• (•OH)	ROS — most reactive radical	Extremely short (nanoseconds)	Fenton/Haber-Weiss reaction
Peroxyl radical	ROO•	Lipid-based radical	Seconds	Lipid peroxidation (propagation)
Alkoxyl radical	RO•	Lipid-based radical	Microseconds	Lipid peroxidation
Thiyl radical	RS•	Sulfur radical	Microseconds	Protein oxidation
Nitric oxide radical	NO•	RNS — radical	Seconds	eNOS, iNOS, nNOS
Nitrogen dioxide radical	NO_2•	RNS — radical	Milliseconds	Reaction of NO with $O_2^{\bullet -}$
Carbonate radical	$CO_3^{\bullet -}$	Carbon radical	Microseconds	Peroxynitrite + CO_2

⚠ Exam Trick

•OH (Hydroxyl radical) is the MOST REACTIVE and MOST DAMAGING free radical in biological systems. $O_2^{\bullet -}$ (Superoxide) is the PRIMARY free radical produced in cells but is far less reactive than •OH. Superoxide is converted to •OH via the Fenton reaction. This hierarchy — $O_2^{\bullet -} \rightarrow H_2O_2 \rightarrow$ •OH — is the most commonly tested concept!

Non-Radical Reactive Species — Important Distinction

Not all Reactive Oxygen Species (ROS) are free radicals. Some ROS are **non-radical oxidising agents** that are equally damaging:

Non-Radical ROS	Symbol	Source	Reactivity / Damage
Hydrogen peroxide	H_2O_2	SOD reaction, ETC, oxidases	Not a radical; crosses membranes; → •OH via Fenton
Singlet oxygen	$^1O_2$	Photosensitisation, myeloperoxidase	Highly reactive excited O_2; damages DNA, lipids
Hypochlorous acid	HOCl	Myeloperoxidase + H_2O_2 + Cl^-	Oxidises proteins, kills bacteria (immune)
Ozone	O_3	Environmental, ozone layer	Damages airway epithelium, oxidises lipids
Peroxynitrite	$ONOO^-$	NO• + $O_2•^-$ reaction (rapid)	Nitrates tyrosine residues in proteins; DNA damage
Hydrogen peroxide (also)	H_2O_2	Enzymatic oxidations (GOX, XO)	Substrate for •OH production (Fenton)

Remember

Mnemonic for non-radical ROS: 'HiSHOP' → H_2O_2 (Hydrogen peroxide), $^1O_2$ (Singlet oxygen), HOCl (Hypochlorous acid), $ONOO^-$ (Peroxynitrite). These four are the non-radical ROS you MUST know for exams.

REACTIVE OXYGEN SPECIES (ROS) — CLASSIFICATION & PROPERTIES

Reactive Oxygen Species (ROS)	A collective term for oxygen-derived small molecules that are highly chemically reactive, including both free radicals ($O_2•^-$, •OH, ROO•) and non-radical species (H_2O_2, $^1O_2$, HOCl, $ONOO^-$). ROS are produced as byproducts of normal metabolism and by external triggers.

The ROS Family Tree — Complete Classification

Category	Species	Formula	Property	Primary Scavenger
Primary ROS (radical)	Superoxide anion radical	$O_2•^-$	Produced first in ETC; precursor to all other ROS	Superoxide Dismutase (SOD)

Category	Species	Formula	Property	Primary Scavenger
Primary ROS (radical)	Hydroxyl radical	•OH	Most reactive biological radical; no specific scavenger	Vitamin C, Mannitol (non-specific)
Secondary ROS (radical)	Peroxyl radical	ROO•	Chain carrier in lipid peroxidation	Vitamin E (chain-breaking)
Secondary ROS (radical)	Alkoxyl radical	RO•	Generated from lipid hydroperoxides	Vitamin E
Non-radical ROS	Hydrogen peroxide	H_2O_2	Membrane permeable; Fenton reaction substrate	Catalase, GPx
Non-radical ROS	Singlet oxygen	$^1O_2$	Excited state O_2; photo-oxidation	Carotenoids (β-Carotene, Lycopene)
Non-radical ROS	Hypochlorous acid	HOCl	Produced by neutrophils (antimicrobial)	Methionine, Taurine
Reactive Nitrogen Species	Nitric oxide radical	NO•	Signaling molecule; vasodilator; → peroxynitrite	Haemoglobin
Reactive Nitrogen Species	Peroxynitrite	$ONOO^-$	From NO + $O_2^{\bullet -}$; nitrates tyrosine residues	Uric acid, Vitamin C

Superoxide Anion Radical ($O_2^{\bullet -}$) — The Primary ROS

Superoxide ($O_2^{\bullet -}$) is the **first and primary ROS** produced in cells. It is generated by the **univalent reduction of molecular oxygen ($O_2 + e^- \rightarrow O_2^{\bullet -}$)**. Despite being less reactive than •OH, it is the **precursor** to all other ROS through a cascade of reactions.

SUPEROXIDE CASCADE — The ROS Production Chain

STEP 1 (Primary): $O_2 + e^- \rightarrow O_2^{\bullet -}$ (Superoxide anion radical)

[By: Mitochondrial ETC Complex I & III, NADPH Oxidase, Xanthine Oxidase]

STEP 2 (Dismutation): $2\ O_2^{\bullet -} + 2\ H^+ \rightarrow H_2O_2 + O_2$

[Enzyme: Superoxide Dismutase (SOD) — FASTEST enzyme known ($k \approx 10^9\ M^{-1}s^{-1}$)]

STEP 3 (Fenton Reaction): $H_2O_2 + Fe^{2+} \rightarrow \bullet OH + OH^- + Fe^{3+}$

[Fe^{2+} regenerated: $Fe^{3+} + O_2\bullet^- \rightarrow Fe^{2+} + O_2$ (Haber-Weiss)]

STEP 4 (Net Haber-Weiss): $H_2O_2 + O_2\bullet^- \rightarrow \bullet OH + OH^- + O_2$

[Net reaction: Superoxide + Hydrogen peroxide → Hydroxyl radical]

FINAL RESULT: •OH — the MOST REACTIVE and MOST DAMAGING species

⚠ Exam Trick

The FENTON REACTION ($Fe^{2+} + H_2O_2 \rightarrow \bullet OH + OH^- + Fe^{3+}$) and HABER-WEISS REACTION ($O_2\bullet^- + H_2O_2 \rightarrow \bullet OH + OH^- + O_2$) are CORE EXAM QUESTIONS. Learn both reactions exactly. point: Fenton requires IRON (Fe^{2+}); Haber-Weiss is the NET reaction combining Fenton + superoxide reduction of Fe^{3+}. These reactions explain WHY iron overload is dangerous!

PRODUCTION OF FREE RADICALS IN CELLS

Free radicals are produced by both **endogenous (metabolic)** and **exogenous (environmental)** sources. Understanding the SITE, ENZYME, and RADICAL PRODUCED is essential for full exam marks.

ENDOGENOUS Sources of ROS

▸ A. Mitochondrial Electron Transport Chain (ETC) — MAJOR SOURCE (70–90% of cellular ROS)

The mitochondrial ETC produces **ATP via oxidative phosphorylation**. During electron transfer, 1–3% of electrons **'leak' from Complex I and Complex III** and react directly with O_2 → Superoxide ($O_2\bullet^-$).

MITOCHONDRIAL ROS PRODUCTION

Complex I (NADH dehydrogenase): $NADH + H^+ + \frac{1}{2}O_2 \rightarrow NAD^+ + H_2O$

→ Electron leak from CoQ site → $O_2 + e^- \rightarrow O_2\bullet^-$ (superoxide, released into MATRIX)

Complex III (Cytochrome bc1): Ubiquinol oxidation

→ Semiquinone radical ($CoQ\bullet^-$) + $O_2 \rightarrow O_2\bullet^-$ (released into INTERMEMBRANE SPACE)

Points:

- Complex I: Superoxide released into MATRIX
- Complex III: Superoxide released into both MATRIX and INTERMEMBRANE SPACE
- Higher membrane potential (proton gradient) → more ROS ('electron pressure')
- Caloric restriction → lowers membrane potential → LESS ROS (longevity mechanism)

▸ B. NADPH Oxidase (NOX) System

NOX (NADPH Oxidase) enzymes are **membrane-bound enzyme complexes** that DELIBERATELY produce superoxide as their primary product — the body's intentional ROS generator for immune defense and cellular signaling.

- Reaction: $NADPH + 2O_2 \rightarrow NADP^+ + H^+ + 2O_2^{\bullet -}$
- Primary cell types: Neutrophils, macrophages (immune cells) — NOX2 subtype ('Respiratory Burst' = 'Oxidative Burst').
- Respiratory Burst: 10–20-fold increase in O_2 consumption → massive $O_2^{\bullet -}$ → converted to H_2O_2 → HOCl (by myeloperoxidase) → KILLS BACTERIA. This is the PRIMARY mechanism of neutrophil killing.
- NOX subtypes: NOX1 (colon), NOX2 (phagocytes — immune), NOX3 (inner ear), NOX4 (kidney/heart — constitutive), NOX5 (sperm).

▸ C. Xanthine Oxidase (XO)

Xanthine oxidase converts **hypoxanthine → xanthine → uric acid** while producing superoxide and H_2O_2. Particularly important in **ischaemia-reperfusion injury (IRI)** — when blood flow is restored after ischaemia, XO activity surges producing a burst of ROS that damages tissue.

- Reaction: $Hypoxanthine + H_2O + O_2 \rightarrow Xanthine + O_2^{\bullet -} + H_2O_2$
- Reaction: $Xanthine + H_2O + O_2 \rightarrow Uric\ acid + O_2^{\bullet -} + H_2O_2$
- Clinical relevance: Gout treatment target (Allopurinol inhibits XO). IRI in heart attack, stroke, organ transplantation.

▸ D. Cytochrome P450 (CYP450) Enzymes

- Located in ER (smooth ER) of hepatocytes. During drug metabolism, electron transfer to CYP450 can 'leak' → $O_2^{\bullet -}$ production.
- Drugs metabolized by CYP450: paracetamol (NAPQI radical), CCl_4 (trichloromethyl radical $CCl_3^{\bullet}$) — hepatotoxic.
- CYP2E1 particularly prone to ROS generation (ethanol metabolism — alcoholic liver disease).

▸ E. Other Endogenous Sources

Source	Enzyme/Process	ROS Produced	Physiological Context
Peroxisomes	Fatty acid β-oxidation, amino acid oxidases	H_2O_2 (primary)	Scavenged by peroxisomal catalase; leakage causes oxidative stress
Lipoxygenase (LOX)	Arachidonic acid metabolism	Lipid radicals (LOO•, LO•)	Inflammation, leukotriene synthesis
Cyclooxygenase (COX)	Prostaglandin synthesis	LOO•	Inflammation; NSAID target
Myeloperoxidase (MPO)	$H_2O_2 + Cl^- \rightarrow HOCl$	HOCl (hypochlorous acid)	Neutrophil killing; also damages host tissue
Monoamine Oxidase (MAO)	Monoamine catabolism (dopamine, serotonin)	H_2O_2	Brain: oxidative stress in Parkinson's — MAO-B inhibitors (Selegiline) protect
Auto-oxidation of small molecules	Adrenalin, Hb, catecholamines	$O_2^{\bullet -}$, H_2O_2	Spontaneous — metal-catalysed

◆ 3.2 EXOGENOUS Sources of Free Radicals

Exogenous Source	Free Radical Produced	Mechanism	Health Consequence
Cigarette smoking	•OH, NO•, NO_2•, organic radicals	Each puff = 10^{15}–10^{16} radicals; depletes Vit C & E	Lung cancer, COPD, CVD, premature ageing
UV radiation (UVA/UVB)	•OH, singlet $^1O_2$, peroxyl ROO•	Photolysis of H_2O, photosensitisation of skin chromophores	Skin cancer (melanoma), photoageing, cataracts
Ionising radiation (X-ray, γ-ray)	•OH (PRIMARY), $O_2^{\bullet -}$	Radiolysis of water: $H_2O \rightarrow$ •OH + H•	DNA strand breaks → cancer, radiation sickness
Air pollutants (O_3, NO_2, PM2.5)	O_3 itself reactive; NO_2•, quinone radicals	Direct reaction with biological molecules; PM activates NOX	Respiratory disease, cardiovascular disease

Exogenous Source	Free Radical Produced	Mechanism	Health Consequence
Xenobiotics / Drugs	CCl_3• (CCl_4), NAPQI (paracetamol), paraquat O_2•$^-$	CYP450 metabolism of toxins; redox cycling	Liver toxicity, kidney damage
Heavy metals (Fe, Cu, Cd, As)	•OH (via Fenton/Haber-Weiss)	Fe^{2+} and Cu^+ catalyse Fenton reaction; Cd displaces Zn in antioxidant enzymes	Multi-organ damage, carcinogenesis
Alcohol (ethanol)	•OH, acetaldehyde radical, 1-hydroxyethyl radical	CYP2E1 metabolism of ethanol → ROS; Fenton reaction (iron accumulation)	Alcoholic liver disease, pancreatitis, brain damage
High-fat diet / Hyperglycaemia	O_2•$^-$, ROO•, carbonyl radicals	Lipid peroxidation; glycation of proteins; mitochondrial dysfunction	Metabolic syndrome, diabetes complications, atherosclerosis

DANGER

Cigarette smoke is the SINGLE LARGEST EXOGENOUS source of free radicals in humans — each cigarette puff contains ~10^{15} free radicals in the gas phase alone. It also depletes plasma Vitamin C by ~40% and Vitamin E. This explains the massively elevated cancer and CVD risk in smokers. In exams: always mention cigarette smoke when asked about exogenous free radical sources.

Antioxidant Defence Systems (Overview)

The body has evolved a **multi-tiered antioxidant defence** against ROS. Understanding this is essential context for understanding the DAMAGE that occurs when these systems are overwhelmed (**Oxidative Stress**).

Oxidative Stress	The imbalance between ROS production and antioxidant defence mechanisms, resulting in net oxidative damage to cells, tissues, and DNA. (Sies, 1985)

Antioxidant	Type	Reaction / Mechanism	Location
Superoxide Dismutase (SOD)	Enzymatic — PRIMARY	$2\ O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$	Cytosol (Cu/Zn-SOD), Mitochondria (Mn-SOD), Extracellular (EC-SOD)
Catalase (CAT)	Enzymatic	$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$	Peroxisomes (mainly), cytosol
Glutathione Peroxidase (GPx)	Enzymatic	$H_2O_2 + 2$ GSH $\rightarrow$ GSSG $+ 2\ H_2O$; also lipid hydroperoxides	Cytosol, mitochondria — requires selenium (GPx1)
Glutathione Reductase (GR)	Enzymatic (regenerates GSH)	GSSG + NADPH $\rightarrow$ 2 GSH + $NADP^+$	Cytosol, mitochondria
Thioredoxin Reductase (TrxR)	Enzymatic	Trx-S_2 + NADPH $\rightarrow$ Trx-$(SH)_2$ + $NADP^+$	Cytosol, mitochondria — requires selenium
Vitamin C (Ascorbate)	Non-enzymatic	Scavenges •OH, $O_2^{\bullet -}$, ROO•; regenerates Vitamin E	Aqueous (cytosol, plasma) — water-soluble
Vitamin E (α-Tocopherol)	Non-enzymatic	Chain-breaking antioxidant; scavenges ROO• in membranes	Lipid phase (cell membranes, lipoproteins)
Glutathione (GSH)	Non-enzymatic — thiol	Scavenges •OH, $^1O_2$, reacts with ROOH; cofactor for GPx	Cytosol (2–10 mM — most abundant non-protein thiol)
β-Carotene / Carotenoids	Non-enzymatic	Quench singlet oxygen ($^1O_2$); peroxyl radical scavenger	Lipid phase
Uric acid	Non-enzymatic	Scavenges •OH, HOCl, $ONOO^-$; chelates Fe and Cu	Plasma (major plasma antioxidant in primates)

⚠ Exam Trick

SOD, Catalase, and GPx are the THREE PRIMARY ENZYMATIC antioxidants. SOD converts $O_2^{\bullet -} \rightarrow H_2O_2$; Catalase and GPx both remove H_2O_2 but differ in location. GPx requires SELENIUM (Se) as cofactor — selenium deficiency → GPx deficiency → H_2O_2 accumulation → •OH → oxidative damage. This explains why selenium is an essential trace mineral!

FREE RADICAL DAMAGE TO LIPIDS — LIPID PEROXIDATION

Lipid Peroxidation	The oxidative deterioration of polyunsaturated fatty acids (PUFAs) in biological membranes by free radicals, leading to a self-propagating chain reaction that produces toxic aldehyde products. Also called 'rancidification' in food science.

Lipids — especially **polyunsaturated fatty acids (PUFAs)** with multiple double bonds (linoleic C18:2, arachidonic C20:4, DHA C22:6) — are **prime targets** for free radical attack because the **bis-allylic hydrogens** (C-H bonds adjacent to double bonds) are weakest and most easily abstracted.

Three Phases of Lipid Peroxidation (Chain Reaction)

LIPID PEROXIDATION — CHAIN REACTION MECHANISM

PHASE 1 — INITIATION (Requires external radical source)

Initiator (•OH, ROO•, $^1O_2$) + LH (PUFA) → L• (Carbon-centred radical) + H_2O

The initiator ABSTRACTS a hydrogen atom from bis-allylic C-H of PUFA

L• = carbon radical (on the PUFA backbone)

PHASE 2 — PROPAGATION (Self-perpetuating chain — can repeat 1000s of times!)

Step A: L• + O_2 → LOO• (Peroxyl radical — fast reaction, k ~ 10^8 $M^{-1}s^{-1}$)

Step B: LOO• + LH → LOOH (Lipid hydroperoxide) + L•

[L• → new round of Step A → LOO• → LOOH → L• ... chain continues]

This is the PROPAGATION CYCLE — one initiating radical damages thousands of PUFAs!

PHASE 3 — TERMINATION (End of chain reaction)

LOO• + LOO• → LOOL + O_2 (non-radical product)

LOO• + Vitamin E (Toc-H) → LOOH + Toc• (Vitamin E TERMINATES the chain!)

Toc• + Vitamin C → Toc-H + Asc• (Vitamin C REGENERATES Vitamin E)

Chain stops when two radicals react or antioxidant intervenes.

⚠ Exam Trick

In lipid peroxidation: INITIATION needs an external radical; PROPAGATION is self-sustaining (L• → LOO• → LOOH → L• cycle, can repeat THOUSANDS of times!); TERMINATION requires chain-breaking antioxidant (Vitamin E) or two radicals meeting. Vitamin E is the ONLY lipid-phase chain-breaking antioxidant. Draw this chain reaction in 10-mark answers — always fetches extra marks!

Products of Lipid Peroxidation & Their Significance

Product	Full Name	Source PUFA	Significance / Disease Link
LOOH	Lipid hydroperoxides	Any PUFA	Primary intermediate; relatively stable; can reform radicals via Fenton
MDA	Malondialdehyde	C20:4 arachidonic acid, DHA	Most abundant LPO product; mutagenic (DNA adducts); TBARS assay biomarker — M2dG adduct causes G→T transversions
4-HNE	4-Hydroxynonenal	Linoleic acid (C18:2 n-6)	Most CYTOTOXIC LPO product; forms protein adducts (Michael addition on Cys, His, Lys); inhibits mitochondrial ETC
Acrolein	Prop-2-enal	C18:2, C18:3	Highly reactive vinyl aldehyde; DNA adducts (γ-OH-PdG); found in cigarette smoke; implicated in neurodegeneration
Isoprostanes (F_2-IsoPs)	F_2-Isoprostanes	Arachidonic acid (C20:4)	GOLD STANDARD biomarker of lipid peroxidation IN VIVO; more stable than MDA; not affected by diet; elevated in CVD, diabetes, smoking

Product	Full Name	Source PUFA	Significance / Disease Link
Oxo-LDL	Oxidised LDL	LDL membrane PUFAs	Central to atherosclerosis: OxLDL taken up by macrophages → foam cells → atherosclerotic plaque
Cholesterol oxidation products	Oxysterols (7-ketocholesterol)	Cholesterol (LDL)	Cytotoxic; atherosclerosis; Alzheimer's disease

★ Point

For exam biomarkers: MDA (measured by TBARS assay) = most commonly used lab marker of lipid peroxidation. But F_2-Isoprostanes (urinary 8-iso-PGF2α) = GOLD STANDARD (most specific, reliable). 4-HNE = most cytotoxic LPO product. Three different questions, three different answers!

Consequences of Lipid Peroxidation on Membranes

- Membrane fluidity ↓ — lipid peroxidation of PUFA side chains → shortened, rigid chains → loss of membrane fluidity and function.
- Membrane permeability ↑ — structural disruption → ions and molecules leak across membrane.
- Membrane protein damage — MDA and 4-HNE form adducts with membrane proteins → inactivation of enzymes, receptors, ion channels.
- Mitochondrial membrane damage → ETC dysfunction → more ROS production (vicious cycle).
- Cell death (apoptosis/necrosis) — severe lipid peroxidation → FERROPTOSIS (iron-dependent regulated cell death characterised by lipid peroxidation).
- Atherosclerosis — LDL oxidation (Ox-LDL) → macrophage foam cell formation → plaque.

Clinical

FERROPTOSIS is a newly recognized regulated cell death pathway driven by lipid peroxidation and iron — distinct from apoptosis and necrosis. GPx4 (Glutathione Peroxidase 4) is the ONLY enzyme that reduces lipid hydroperoxides in membranes — its inhibition causes ferroptosis. This is highly relevant in cancer therapy (inducing ferroptosis in tumour cells).

FREE RADICAL DAMAGE TO PROTEINS

Proteins are critical cellular machinery — enzymes, receptors, structural proteins, transport proteins. Free radicals attack proteins at **amino acid side chains**, the **peptide backbone**, and **sulfhydryl (-SH) groups**, leading to loss of structure and function.

Types of Protein Oxidation

A. Protein Carbonylation (PRIMARY and MOST STUDIED marker)

Free radicals (especially •OH) attack amino acid side chains, introducing **carbonyl groups (-C=O: aldehydes and ketones)**. Most susceptible amino acids: **Proline, Arginine, Lysine, Threonine** (PALT mnemonic). Protein carbonyls are the **PRIMARY BIOMARKER of protein oxidative damage** — measured by DNPH (dinitrophenylhydrazine) assay → OTC (ozone treatment)/DNP method.

PROTEIN CARBONYLATION MECHANISM

•OH + Protein-H → Protein• (carbon-centred protein radical) + H_2O

Protein• + O_2 → Protein-OO• (protein peroxyl radical)

Protein-OO• + H → Protein-OOH (protein hydroperoxide)

Protein-OOH → Protein=O (CARBONYL — aldehyde/ketone) via β-scission or metal-catalysed reduction

Specific examples:

Proline → Glutamic semialdehyde (carbonyl product)

Arginine → Glutamic semialdehyde

Lysine → Aminoadipic semialdehyde

Threonine → 2-Amino-3-ketobutyric acid

B. Protein Nitration — Tyrosine Nitration

Peroxynitrite ($ONOO^-$), formed from **NO• + O_2•$^-$**, nitrates **tyrosine residues** → 3-Nitrotyrosine (3-NT) — a specific biomarker of peroxynitrite-mediated damage and nitrosative stress.

- Reaction: $ONOO^-$ + Tyr-OH → 3-Nitrotyrosine (3-NT) + H_2O
- Consequences: Nitration at Tyr → loss of phosphorylation site → disruption of signal transduction; Inactivation of enzymes (Mn-SOD tyrosine nitration → loss of antioxidant defence).
- 3-Nitrotyrosine is elevated in: Alzheimer's disease, Parkinson's disease, atherosclerosis, ALS, sepsis, ARDS.

C. Thiol Oxidation — Sulfhydryl Group Attack

Cysteine (-SH) and Methionine (thioether) residues are **most reactive** with ROS due to sulfur atom's electron-rich nature.

Oxidation Product	Reaction	Reversibility	Consequence
Protein disulfide (P-S-S-P)	2 Cys-SH + oxidant → Cys-S-S-Cys	REVERSIBLE — reduced by Trx/Grx	Intermolecular cross-linking, conformational change
Protein mixed disulfide (S-glutathionylation)	Cys-SH + GSSG → Cys-S-S-G + GSH	REVERSIBLE — regulatory modification	Protects Cys from over-oxidation; signal transduction
Sulfinic acid (-SO_2H)	Cys-SH + 2[O] → Cys-SO_2H	Partially reversible (via Sulfiredoxin)	Enzyme inactivation
Sulfonic acid (-SO_3H)	Cys-SH + 3[O] → Cys-SO_3H	IRREVERSIBLE	Complete enzyme inactivation; protein aggregation
Methionine sulfoxide	Met-S- + [O] → Met-S=O	REVERSIBLE — MsrA/MsrB	Loss of hydrophobicity; conformational change

▸ D. Protein Cross-linking and Fragmentation

- Protein-Protein Cross-links: Two carbonyl-containing proteins react → intermolecular cross-links → HIGH molecular weight aggregates (difficult to degrade by proteasome).
- Protein-DNA Cross-links: Protein radicals react with DNA → 'stuck' complexes → replication block.
- Backbone Cleavage (β-scission): •OH attacks α-carbon of peptide backbone → protein fragments.
- Aggregate Formation: Accumulation of oxidised, cross-linked protein aggregates → neurodegeneration (Amyloid in AD, α-synuclein in PD, Tau in AD, Huntingtin in HD).

Consequences of Protein Oxidation — Clinical Relevance

Consequence	Mechanism	Disease/Condition
Enzyme inactivation	Active site residue oxidised → loss of catalytic activity	All age-related diseases
Receptor dysfunction	Receptor binding domain oxidised → impaired signalling	Insulin resistance, neurodegeneration
Structural protein damage	Collagen cross-linking (skin ageing); elastin oxidation (lung emphysema)	Ageing, COPD, atherosclerosis
Proteasomal overload	Oxidised proteins must be degraded — mildly oxidised → proteasome; severely	Protein aggregate diseases (Alzheimer's, Parkinson's, cataracts)

Consequence	Mechanism	Disease/Condition
	oxidised → insoluble aggregate → evades degradation	
Antioxidant enzyme inactivation	Mn-SOD nitration → ↓ activity → MORE ROS (vicious cycle)	Accelerated oxidative stress — mitochondrial disease
Lens protein aggregation	Crystallin protein oxidation → protein aggregates in lens → CATARACT	Cataract (most common cause of blindness globally)

⚠ Exam Trick	BIOMARKER SUMMARY for protein oxidation: (1) Protein Carbonyls — measured by DNPH assay — MOST COMMONLY USED biomarker; (2) 3-Nitrotyrosine — biomarker of peroxynitrite/nitrosative stress; (3) Dityrosine — cross-linking biomarker. Learn all three — examiners ask 'name a biomarker of protein oxidative damage' regularly.

FREE RADICAL DAMAGE TO CARBOHYDRATES

Carbohydrates undergo free radical-mediated oxidation through **two major pathways**: (1) Direct radical oxidation, and (2) **Non-enzymatic Glycation** (Maillard reaction in vivo), which produces **Advanced Glycation End Products (AGEs)** — a process **potentiated by oxidative stress**, called **Glycoxidation**.

Direct Radical Attack on Carbohydrates

Hydroxyl radicals (•OH) attack carbohydrate molecules by **hydrogen abstraction** → carbon-centred radical → ring-opening reactions → oxidation products.

- •OH attacks C-H bonds of monosaccharides (glucose, ribose, deoxyribose) → carbonyl compounds and dehydration products.
- Deoxyribose in DNA backbone is particularly susceptible → strand breaks (covered in Section 7).
- Products of glucose oxidation: Gluconolactone, gluconic acid, glyoxal, methylglyoxal (MGO) — all highly reactive carbonyl compounds.
- Hyaluronic acid (glycosaminoglycan) depolymerisation: •OH cleaves HA backbone → shorter chains → loss of viscosity in synovial fluid (rheumatoid arthritis).

Non-Enzymatic Glycation — The Maillard Reaction In Vivo

GLYCATION CASCADE — Glucose + Protein → AGEs

STEP 1 — EARLY GLYCATION (Schiff Base Formation):

Reducing sugar (Glucose) + Free amino group (Lys, N-terminus) → Schiff Base (aldimine)

[Reversible — occurs in minutes to hours]

STEP 2 — AMADORI REARRANGEMENT:

Schiff Base → Amadori Product (ketoamine)

e.g., Glucose + Haemoglobin → HbA1c (Glycosylated Haemoglobin — Amadori product)

[Relatively stable — hours to weeks; REVERSIBLE with normal glucose]

STEP 3 — ADVANCED GLYCATION END PRODUCTS (AGEs):

Amadori Products undergo complex dehydration, condensation, oxidation → IRREVERSIBLE AGEs

Examples: CML (Nε-Carboxymethyllysine), GOLD (glyoxal-lysine dimer), Pentosidine

[IRREVERSIBLE — months to years; accumulate on long-lived proteins: collagen, lens]

POINT: HbA1c = Amadori product (NOT an AGE, but reflects 3-month average glucose)

⚠ Exam Trick	HbA1c is an AMADORI PRODUCT — NOT an AGE (Advanced Glycation End Product). Examiners deliberately use this to test depth of understanding. HbA1c = Glucose + Haemoglobin β-chain N-terminal valine → Schiff base → Amadori product. It reflects 3-month average blood glucose because RBC lifespan = 90–120 days. AGEs form on longer-lived proteins like collagen (lifespan = years).

Glycoxidation — ROS + Glycation = Double Damage

Glycoxidation	The oxidative modification of glycated proteins — the combination of glycation (high glucose) and oxidative stress (ROS) producing highly toxic reactive carbonyl compounds and AGEs. glycoxidation products: methylglyoxal (MGO), glyoxal, 3-deoxyglucosone.

- Methylglyoxal (MGO): most reactive dicarbonyl; reacts with arginine, lysine → MG-H1 (methyl glyoxal-derived hydroimidazolone) — major AGE in diabetics.
- Glyoxal: similar to MGO; forms GOLD adducts on proteins.

- Glycoxidation produces AGEs FASTER than glycation alone — explaining why diabetics with poor glycaemic control have exponentially more tissue damage.

AGE Receptors (RAGE) and Disease Mechanisms

AGEs bind to **RAGE (Receptor for Advanced Glycation End Products)** → activates **NF-κB** → proinflammatory cytokines (TNF-α, IL-6, IL-1β) → **CHRONIC INFLAMMATION** + MORE ROS → accelerated damage.

Tissue / Protein Affected	AGE Effect	Clinical Consequence
Collagen (connective tissue)	Cross-linking → loss of elasticity	Arterial stiffness, joint rigidity, atherosclerosis, reduced wound healing
Lens crystallins (eye)	Aggregation → clouding of lens	DIABETIC CATARACTS (senile cataracts in non-diabetics too)
Glomerular basement membrane (kidney)	Thickening, cross-linking → altered charge/permeability	Diabetic nephropathy — proteinuria, renal failure
Myelin (nerve)	Demyelination, conduction slowing	Diabetic neuropathy — numbness, pain, autonomic dysfunction
Retinal vessels	VEGF ↑ (via RAGE/NF-κB), microaneurysm formation	Diabetic retinopathy — leading cause of blindness in adults
LDL / Apolipoproteins	AGE-modified LDL not recognised by LDL receptor → cleared by scavenger receptors → foam cells	Accelerated atherosclerosis in diabetes
Haemoglobin (HbA1c)	Amadori product only (not AGE); reduces O_2-carrying capacity	HbA1c >6.5% → T2DM diagnosis; >8% → poor control

Clinical

RAGE (Receptor for Advanced Glycation End Products) is a pattern recognition receptor of the immunoglobulin superfamily expressed in endothelial cells, macrophages, and neurons. RAGE-AGE interaction activates NF-κB → perpetual inflammatory cycle. Anti-RAGE therapy is an emerging target in diabetes, Alzheimer's (AGEs found in amyloid plaques), and

atherosclerosis. Soluble RAGE (sRAGE) acts as a DECOY receptor — protective.

FREE RADICAL DAMAGE TO NUCLEIC ACIDS (DNA & RNA)

DNA is the most critical target of free radical damage because **unrepaired DNA damage leads to MUTATION → CANCER or CELL DEATH**. •OH is the primary culprit — it attacks both the **sugar-phosphate backbone** and the **nitrogenous bases**. Over **20,000 oxidative lesions per cell per day** are estimated to occur in a human cell under normal conditions.

Types of Oxidative DNA Damage

Type of Damage	Mechanism	Product	Mutagenic Consequence
Base oxidation (Guanine — MOST susceptible)	•OH + Guanine → 8-OHdG (8-hydroxy-2'-deoxyguanosine)	8-OHdG / 8-oxoGua	G→T transversion mutations (8-OHdG pairs with adenine instead of cytosine)
Base oxidation (Thymine)	•OH + Thymine → Thymine glycol	Thymine glycol (Tg)	Blocks DNA polymerase → replication arrest
Cytosine deamination	•OH/RNS → 5-hydroxycytosine	5-hydroxycytosine	C→T transition mutations
Adenine oxidation	•OH + Adenine → 8-hydroxyadenine	8-Hydroxyadenine (8-OHA)	A→C transversions
Single strand break (SSB)	•OH attacks deoxyribose (C4' hydrogen abstraction) → strand break	SSB (nick in one strand)	Blocked replication; if unrepaired → DSB
Double strand break (DSB)	Two SSBs within ~10 bp of each other; or ionising radiation direct effect	DSB	MOST LETHAL DNA lesion → chromosomal rearrangements, cell death
DNA-protein cross-links	Protein radical + DNA radical → covalent bond	DPC (DNA-Protein Crosslink)	Blocks replication and transcription

Type of Damage	Mechanism	Product	Mutagenic Consequence
DNA-DNA cross-links	Inter/intrastrand crosslinks	ICL (Interstrand crosslink)	Blocks strand separation → replication failure

8-OHdG — The Primary Biomarker of Oxidative DNA Damage

8-OHdG	8-Hydroxy-2'-deoxyguanosine — the oxidative modification product of guanine in DNA, formed by •OH attack at C-8 of guanine. The GOLD STANDARD and MOST WIDELY USED biomarker of oxidative DNA damage. Measured in urine (as repair product), leukocytes, or tissue by ELISA or HPLC-ECD.

FORMATION AND CONSEQUENCES OF 8-OHdG

FORMATION:

•OH + Guanine (in DNA) → C8-OH adduct radical → 8-OHdG (or 8-oxo-7,8-dihydroguanine = 8-oxoG)

MISPAIRING (MUTAGENIC):

Normal: G pairs with C (Watson-Crick: G-C)

8-OHdG: 8-OHdG pairs with A (anti/syn conformer) → G→T TRANSVERSION MUTATION

DNA Polymerase inserts A opposite 8-OHdG → at next replication: A pairs with T

RESULT: G:C → T:A transversion (most common mutation in human cancer)

REPAIR:

OGG1 (8-oxoguanine DNA glycosylase) — BASE EXCISION REPAIR enzyme

OGG1 removes 8-OHdG → repaired by BER pathway

Urinary 8-OHdG = measure of both DAMAGE and REPAIR rate

ELEVATED in: Cancer, diabetes, CVD, neurodegenerative diseases, smokers, obesity

⚠ Exam Trick

8-OHdG is elevated in urine, and this elevation is used as a biomarker for cancer risk assessment and oxidative stress measurement. The mutation it causes is G→T TRANSVERSION (not transition!). Transversion =

purine↔pyrimidine change (G→T). This mutation is found in codon 12 of K-RAS in lung cancer (smoking-related). Very high-yield exam integration point!

DNA Damage Repair Systems

Repair Pathway	Lesion Repaired	Enzyme	Consequence of Failure
Base Excision Repair (BER)	Oxidised bases (8-OHdG, thymine glycol, AP sites)	OGG1, MUTYH, PARP, DNA ligase III	Accumulation of oxidised bases → mutations, cancer
Nucleotide Excision Repair (NER)	Bulky adducts, UV photoproducts, some oxidative crosslinks	XPC, XPA, ERCC1/XPF, PCNA	Xeroderma Pigmentosum (XP) — extreme UV sensitivity → skin cancer
Mismatch Repair (MMR)	Mismatched bases (8-OHdG:A mispairs)	MSH2, MLH1, PMS2	Lynch Syndrome (hereditary colorectal cancer) when mutated
Homologous Recombination (HR)	Double strand breaks (DSBs) — accurate	BRCA1, BRCA2, RAD51	BRCA1/2 mutation → breast/ovarian cancer
Non-Homologous End Joining (NHEJ)	Double strand breaks — error-prone	Ku70/Ku80, DNA-PK, DNA ligase IV	Chromosomal rearrangements, translocations → cancer

Long-term Consequences of DNA Oxidative Damage

- **MUTAGENESIS**: Unrepaired 8-OHdG → G→T transversion → proto-oncogene activation (K-RAS, BRAF) or tumour suppressor inactivation (p53, RB).
- **CARCINOGENESIS:** Accumulation of mutations in cell cycle regulatory genes → dysregulated proliferation → cancer. DNA oxidative damage is mechanistically linked to ALL major human cancers.
- **AGEING:** Mitochondrial DNA (mtDNA) is 10× more susceptible to oxidative damage than nuclear DNA (no histones, closer to ETC ROS source, limited repair) → mtDNA mutations accumulate with age → mitochondrial dysfunction → 'Mitochondrial Theory of Ageing' (Harman, 1972).
- **NEURODEGENERATION:** Post-mitotic neurons cannot replace themselves; accumulating DNA damage → neuronal death → Alzheimer's (8-OHdG elevated), Parkinson's, ALS.
- **TELOMERE SHORTENING:** Telomeres (TTAGGG repeats) are particularly sensitive to oxidative damage (guanine-rich). Oxidative stress → accelerated telomere attrition → premature cellular senescence → tissue ageing.

Remember

The 'FREE RADICAL THEORY OF AGEING' (Harman, 1956): Ageing and age-related diseases result from accumulated oxidative damage to cells and

tissues by free radicals produced during normal metabolism. This theory underpins the entire nutraceutical antioxidant industry. Later refined as the 'MITOCHONDRIAL FREE RADICAL THEORY OF AGEING' — mtDNA damage is the primary driver.

DIETARY FIBRES AS FUNCTIONAL FOOD INGREDIENTS

Dietary Fibre	The edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the large intestine. Includes: polysaccharides, oligosaccharides, lignin, and associated plant substances. (AACC International, 2001; Codex Alimentarius, 2009)

The characteristics of dietary fibre: **(1) Resistant to human digestive enzymes**; (2) Fermented (partially or fully) by colonic microbiota; (3) Produces **Short Chain Fatty Acids (SCFA)** — acetate, propionate, butyrate; (4) Provides **physiological benefits** beyond nutrition.

Classification of Dietary Fibre

Classification Basis	Category	Examples	Property
Solubility	SOLUBLE FIBRE	Pectin, Beta-glucan, Guar gum, Psyllium (partly), Inulin/FOS	Forms viscous gel in gut; fermented; LDL ↓; glycemia ↓
Solubility	INSOLUBLE FIBRE	Cellulose, Hemicellulose (most), Lignin, Wheat bran AX	Bulks stool; speeds transit; not fermented; constipation relief
Fermentability	HIGHLY FERMENTABLE	Pectin, Inulin, FOS, Beta-glucan	Extensive SCFA production; prebiotic effect
Fermentability	PARTIALLY FERMENTABLE	Psyllium, Guar gum, some Hemicellulose	Moderate SCFA; some stool bulk
Fermentability	NON-FERMENTABLE / SLOWLY FERMENTED	Cellulose, Lignin, Resistant Starch (RS1)	Primarily mechanical/physical effect

Classification Basis	Category	Examples	Property
Viscosity	HIGH-VISCOSITY	Beta-glucan, Guar gum, Pectin	Viscosity = cholesterol and glycemic effect
Viscosity	LOW-VISCOSITY	Wheat bran, Cellulose	Bulk forming; less metabolic effect

⚠ Exam Trick

Soluble vs Insoluble fibre is THE most tested fibre concept: SOLUBLE = dissolves in water, forms gel, lowers cholesterol and blood glucose, mostly fermented (Examples: pectin, β-glucan, psyllium husk, inulin). INSOLUBLE = does not dissolve, increases stool bulk, speeds colon transit, prevents constipation and colorectal cancer (Examples: cellulose, lignin, wheat bran). Psyllium = BOTH soluble (husk mucilage) and insoluble (seed coat) — trick question!

Individual Dietary Fibres — Detailed Profiles

Cellulose

Source	Cell walls of ALL plant foods — cotton, wood pulp, whole grains, vegetables, fruits, legumes. Most abundant organic polymer on Earth.
Chemical Nature	Linear polymer of glucose units linked by β(1→4) glycosidic bonds. Degree of Polymerisation (DP) = 300–15,000 glucose units. Highly crystalline and insoluble — hydrogen bonding between chains creates rigid microfibrils. MW: 50,000–500,000 Da. Chemical formula of repeating unit: $(C_6H_{10}O_5)n$.
Fiber Type (Sol./Insol.)	INSOLUBLE (non-fermentable)
Mechanism	Resists human enzymes (no β-glucosidase). Absorbs water (2-3× its weight) → stool bulk ↑. Colonic transit time ↓. Minimal fermentation → minimal SCFA. Physical scrubbing of intestinal wall.
Health Benefits	Constipation relief and prevention; Diverticular disease prevention; Colorectal cancer prevention (transit time ↓, carcinogen dilution); Satiety (bulky food); Weight management.

Pectin

Source	Apple pomace (peel and core) — RICHEST commercial source; Citrus peel (lemon, orange, grapefruit — 15–30% dry weight); Apricot, quince, beet. Commercial food additive (E440) — used as gelling agent in jams.
Chemical Nature	Complex polysaccharide — backbone of α(1→4)-linked D-galacturonic acid units (partially methyl-esterified). Degree of Methyl-Esterification (DM): High-methyl pectin (DM >50%) = gels in high sugar/acid; Low-methyl pectin (DM <50%) = gels with Ca^{2+}. Side chains: Arabinogalactan, rhamnosyl residues. MW: 50,000–800,000 Da.
Fiber Type (Sol./Insol.)	SOLUBLE, highly fermentable
Mechanism	Forms viscous gel in upper GI → slows gastric emptying → reduced postprandial glucose (glycemic index ↓). Bile acid sequestration → LDL ↓. Fermented by Bifidobacterium → SCFA (butyrate). Prebiotic effect. Chelates heavy metals (lead, cadmium) → detoxification.
Health Benefits	Cholesterol reduction (LDL ↓ 10–15%); Type 2 Diabetes (glycemic control); Gut health (prebiotic → Bifidobacterium ↑); Heavy metal detoxification (lead chelation); Anti-diarrhoeal; Colorectal cancer prevention; Weight management.

🌾 Beta-Glucan (β-Glucan)

Source	Oats (Avena sativa) — 2.5–8% dry weight; Oat bran — richest (up to 17%); Barley (Hordeum vulgare) — 3–11%; Mushrooms (Lentinan from shiitake — β(1→3)(1→6)-glucan); Yeast (Zymosan); Baker's yeast cell wall.
Chemical Nature	Mixed linkage (1→3),(1→4)-β-D-glucan (oat/barley). Linear glucose polymer. High molecular weight (MW: 50,000–3,000,000 Da) — higher MW = more viscous = greater effect. Oat and barley β-glucan = (1→3) and (1→4) linkages only. Mushroom β-glucan = (1→3) backbone with (1→6) branches (immunomodulatory). WATER-SOLUBLE.
Fiber Type (Sol./Insol.)	SOLUBLE, highly fermentable, HIGH VISCOSITY
Mechanism	Viscous gel in small intestine → bile acid sequestration → hepatic LDL uptake ↑ → LDL ↓. Viscous gel → slows glucose absorption

	→ glycemic response ↓ → insulin demand ↓. Fermented by Lactobacillus/Bifidobacterium → butyrate → colonocyte health. GLP-1 and PYY stimulation → satiety ↑. Mushroom β-glucan → dectin-1 receptor activation on immune cells → NK cell ↑, macrophage activation → anti-tumour immunity.
Health Benefits	Cholesterol reduction — FDA APPROVED HEALTH CLAIM (3g/day oat β-glucan reduces CHD risk); Type 2 Diabetes glycemic control; Weight management (satiety ↑); Immune modulation (mushroom β-glucan → cancer adjuvant); Gut health (prebiotic); Blood pressure reduction.

Psyllium Husk (Isabgol)

Source	Plantago ovata (Blonde Psyllium) seeds — India (Gujarat, Rajasthan) is WORLD'S LARGEST PRODUCER (80% global supply); also P. psyllium (Black psyllium). Husk = seed coat — primary medicinal part.
Chemical Nature	Arabinoxylan: backbone of β(1→4)-D-xylose with arabinose and xylose side chains. HIGHLY viscous mucilage. Two fractions: (1) Water-soluble mucilaginous fraction (~30% — forms gel rapidly in water — primary active fraction); (2) Water-insoluble husk fraction (~70% — bulk forming). Gel forms within 15 minutes of water contact. MW: 50,000–600,000 Da (gel-forming fraction).
Fiber Type (Sol./Insol.)	BOTH soluble (mucilage) + insoluble (seed coat) — DUAL action
Mechanism	Soluble fraction: Viscous gel → slows gastric emptying → postprandial glucose ↓; Bile acid sequestration → LDL ↓ (up to 10–15%); Prebiotic effect. Insoluble fraction: Stool bulk ↑; Colon transit ↑; Constipation relief. BIDIRECTIONAL: Relieves both constipation (insoluble) AND diarrhoea (soluble mucilage absorbs water, normalises stool consistency).
Health Benefits	Constipation (first-line non-pharmacological treatment); Diarrhoea (IBS-D — stool normalising); Cholesterol reduction (FDA approved claim); Type 2 Diabetes (postprandial glucose ↓); IBS (IBS-C and IBS-D); Inflammatory Bowel Disease; Weight management; Haemorrhoids.

Lignin

Source	Woody parts of plants — secondary cell walls of vascular plants; Wheat bran; Flaxseed (high lignan/lignin); Sesame seeds; Mature vegetables. NOT a carbohydrate — unique structural component.
Chemical Nature	NOT a polysaccharide — Lignin is a complex aromatic PHENYLPROPANOID POLYMER made of three monolignol monomers: p-Coumaryl alcohol (H-type), Coniferyl alcohol (G-type — guaiacyl), Sinapyl alcohol (S-type — syringyl). Crosslinked by ether (β-O-4), C-C, and ester bonds. Non-digestible. Non-fermentable. MW: Very high (10,000–100,000s Da). Note: Lignin ≠ Lignan (lignans are polyphenolic dimers; lignin is structural polymer).
Fiber Type (Sol./Insol.)	INSOLUBLE, NON-FERMENTABLE
Mechanism	Physical binding of bile acids in gut (non-specific adsorption) → LDL ↓. Binds potential carcinogens (mutagens, heterocyclic amines from cooked meat) → reduces exposure. Adsorption of oestrogen → enterohepatic recirculation ↓ → may reduce hormone-dependent cancer risk. Antioxidant (phenylpropanoid structure has radical scavenging ability — minor contribution).
Health Benefits	Colorectal cancer prevention (carcinogen binding); Cholesterol reduction (bile acid binding); Hormone-dependent cancer risk reduction (oestrogen binding); Constipation prevention (bulk forming with water absorption).

⚠ Exam Trick	LIGNIN ≠ LIGNAN — two completely different compounds! Lignin = insoluble structural polymer of plant cell walls (NOT a carbohydrate). Lignans = polyphenolic phytoestrogen compounds (e.g., SDG from flaxseed). Examiners deliberately confuse students with this! Also: Lignin is technically NOT a carbohydrate (it's an aromatic polymer) but is classified as dietary fibre because it resists digestion.

Guar Gum

Source	Guar beans (Cyamopsis tetragonoloba) — legume grown in India (Rajasthan is major producer), Pakistan, USA. Extracted from endosperm of guar seeds. India produces 80% of world supply.

Chemical Nature	Galactomannan: β(1→4)-linked D-mannose backbone with α(1→6)-linked D-galactose side chains (Mannose:Galactose ratio ≈ 2:1). High molecular weight: 200,000–300,000 Da. HIGHEST VISCOSITY of all natural gums. Water-soluble. Forms a thick, viscous solution — 8× more viscous than cornstarch.
Fiber Type (Sol./Insol.)	SOLUBLE, partially fermentable, VERY HIGH VISCOSITY
Mechanism	Highest viscosity → strongest slowing of gastric emptying and small intestinal glucose absorption → most potent glycemic control among fibres. Bile acid sequestration → LDL ↓. Satiety hormone release (GLP-1, CCK ↑) → caloric intake ↓. Partially fermented → SCFA production → gut health.
Health Benefits	Type 2 Diabetes — most viscous fibre = best postprandial glucose reduction; Hypercholesterolaemia (LDL ↓ 10–15%); Weight management (satiety ↑); Food industry thickener (E412 — used in dairy, sauces, bakery); Constipation and IBS.

COMPLEX CARBOHYDRATES AS FUNCTIONAL FOOD INGREDIENTS

Complex Carbohydrates	Polysaccharides and oligosaccharides consisting of multiple monosaccharide units linked by glycosidic bonds. They include starch, glycogen, dietary fibre, and resistant starch. Distinguished from simple carbohydrates (mono- and disaccharides) by their longer chains, lower glycemic index, and additional functional properties.

Resistant Starch (RS) — The Functional Starch

Resistant Starch (RS) is the **fraction of starch (and starch degradation products) that escapes digestion in the small intestine** of healthy individuals and reaches the large intestine intact, where it functions as a **prebiotic** and produces SCFA (especially **BUTYRATE**) — the preferred fuel of colonocytes.

RS Type	Classification	Example Foods	Mechanism of Resistance
RS1 (Physically inaccessible)	Starch physically enclosed in intact plant cell walls or protein matrix	Whole grains, seeds, legumes (intact/coarsely milled)	Physical barrier prevents enzyme access; grinding increases digestibility
RS2 (Resistant starch granules)	Compact crystalline (B-type crystallinity) raw starch granules	Raw potato, green (unripe) banana, raw corn, high-amylose maize	Compact B-type crystalline structure resists gelatinisation; cooking destroys resistance
RS3 (Retrograded starch)	Retrograded amylose formed on cooling cooked starch	Cooked-then-cooled potato, cooked-cooled rice/pasta, cornflakes	Linear amylose re-crystallises on cooling → resistant conformation; reheating partially reverses it
RS4 (Chemically modified)	Chemically cross-linked or substituted starch	Hi-maize resistant starch, modified food starches (E1400-E1452)	Cross-links prevent enzyme penetration; chemical substitution blocks hydrolysis sites
RS5 (Amylose-lipid complex)	Amylose complexed with lipid/emulsifier (V-type starch complex)	Extruded starchy foods with added fatty acids/emulsifiers	V-type crystallinity of amylose-lipid inclusion complex resists amylase

★ Point	RS3 (Retrograded starch) is particularly important practically: Cooking potato → hot = mostly digestible starch. Cooking potato then COOLING → RS3 increases significantly (3–4×). Sushi rice (vinegar-added cooled rice), cold pasta salad, day-old boiled potatoes — all have MORE RS3 and lower glycemic index than freshly cooked equivalents. This is a 'common knowledge' exam trick!

Health Benefits of Resistant Starch and Complex Carbohydrates

Health Benefit	Mechanism	Evidence / Clinical Data
Colorectal cancer prevention	RS fermentation → butyrate → colonocyte energy + anti-proliferative (inhibits histone deacetylase → tumour suppressor gene expression ↑) → apoptosis in cancer cells	Strong observational evidence; high-fibre populations (rural Africa) have near-zero colorectal cancer rates

Health Benefit	Mechanism	Evidence / Clinical Data
Type 2 Diabetes prevention / management	Complex carbs → slower glucose absorption → lower GI → reduced postprandial glycemia and insulin demand; RS improves insulin sensitivity (second meal effect)	Meta-analyses: 10g/day increase in fibre → 10% reduced T2DM risk
Weight management	High fibre = low energy density; viscous fibre → gastric emptying delay → GLP-1 ↑, PYY ↑, ghrelin ↓ → satiety ↑ → spontaneous caloric intake ↓	Meta-analyses: 14g/day additional fibre → 10% caloric intake reduction
Cardiovascular disease prevention	Soluble fibre (pectin, β-glucan) → bile acid sequestration → LDL ↓ 10-15%; resistant starch → propionate production → hepatic de novo lipogenesis ↓ → TG ↓	FDA health claim (oat β-glucan); EFSA approved (beta-glucan + LDL)
Gut microbiome modulation (Prebiotic)	RS, pectin, inulin → selectively fermented by Bifidobacterium, Lactobacillus, Faecalibacterium prausnitzii → SCFA; ↑ microbial diversity → ↓ dysbiosis	Strong evidence; RS increases Bifidobacterium and Ruminococcus bromii (RS specialist)
Mineral absorption (Ca, Mg, Zn)	Fermentation → colon acidification (SCFA) → increased solubility of Ca, Mg, Zn → enhanced absorption (particularly in premenopausal women and adolescents)	Short-chain inulin-type fructans → Ca absorption ↑ 20-40%

The Second Meal Effect of Resistant Starch

A unique property of RS is the **'Second Meal Effect' (or 'second meal phenomenon')**: consumption of RS at breakfast **reduces glycemic response at LUNCH** even when lunch contains

regular digestible carbohydrates. Mechanism: RS fermentation at breakfast → propionate produced → propionate inhibits hepatic glucose output → reduced glycemia at next meal.

◆ 9.4 Comparison of Complex Carbohydrates

Carbohydrate	Type	Linkage	Solubility	GI	Function
Starch (digestible)	Polysaccharide	α(1→4) amylose; α(1→4)/(1→6) amylopectin	Insoluble (raw); Soluble (cooked)	High (70-95)	Energy source; NO functional food benefit
Resistant Starch (RS)	Modified polysaccharide	α(1→4) + retrograded/complexed	Insoluble	Low (10-30)	Prebiotic; butyrate; insulin sensitivity
Beta-glucan	Polysaccharide	β(1→3)/(1→4)-glucose	Soluble	Low-Medium	Cholesterol ↓ (FDA claim); glycemia ↓
Pectin	Polysaccharide	α(1→4)-galacturonic acid	Soluble	Low	LDL ↓; glycemic control; prebiotic
Cellulose	Polysaccharide	β(1→4)-glucose	Insoluble	None	Stool bulk; constipation relief
Inulin / FOS	Oligosaccharide/ Polysaccharide	β(2→1)-fructose	Soluble	Very Low (2)	Prebiotic; bifidogenic; Ca absorption ↑
Arabinoxylan (wheat bran)	Heteropolysaccharide	β(1→4)-xylose + arabinose	Insoluble (mainly)	None	Stool bulk; colorectal cancer prevention
Guar gum	Galactomannan	β(1→4)-mannose + α(1→6)-galactose	Soluble	Low	Glycemia ↓ (most viscous); LDL ↓

Recommended Daily Intake of Dietary Fibre

Population Group	WHO/FAO Recommendation	ICMR (India) Recommendation	Average Indian Intake
Adults (general)	≥25g/day	40g/day (higher due to plant-based diet)	20–25g/day (below recommendation)
Type 2 Diabetics	>35g/day (esp. soluble fibre)	45g/day	Often inadequate
Heart disease prevention	>25g/day soluble fibre	35–40g/day	Below target
Children (2–18 years)	Age + 5g/day (US DRI formula)	25–30g/day	10–15g/day (significantly below)
Pregnant women	≥28g/day	30–35g/day	Inadequate in most surveys

Clinical

India's average fibre intake (20–25g/day) is BELOW the ICMR recommendation of 40g/day despite a predominantly plant-based diet — because modern food processing removes fibre (refined wheat flour 'maida' vs whole wheat 'atta'; polished white rice vs brown rice). Promoting whole grains, pulses, and vegetables is the primary public health nutrition strategy to increase fibre intake in India.

DEFINITIONS GLOSSARY —

Free Radical	Any atom or molecule containing one or more unpaired electrons, making it highly reactive and short-lived (nanoseconds). Denoted by • (dot) notation.
Reactive Oxygen Species (ROS)	Collective term for oxygen-derived reactive molecules including both free radicals ($O_2^{\bullet -}$, •OH, ROO•) and non-radical oxidants (H_2O_2, $^1O_2$, HOCl, $ONOO^-$).
Superoxide Anion Radical ($O_2^{\bullet -}$)	Primary ROS produced in cells; formed by univalent reduction of O_2; precursor to all other ROS; scavenged by Superoxide Dismutase (SOD).

Hydroxyl Radical (•OH)	Most reactive biological free radical; formed via Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow$ •OH); half-life in nanoseconds; attacks all biomolecules indiscriminately.
Fenton Reaction	$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} +$ •OH + OH^-. Iron-catalysed conversion of hydrogen peroxide to the highly reactive hydroxyl radical. Explains iron overload toxicity.
Haber-Weiss Reaction	Net reaction: $O_2•^- + H_2O_2 \rightarrow O_2 +$ •OH + OH^-. Combines Fenton reaction + superoxide-mediated Fe^{3+} reduction. Overall converts superoxide + H_2O_2 to hydroxyl radical.
Oxidative Stress	Imbalance between ROS production and antioxidant defence mechanisms, resulting in net oxidative damage to cells. (Sies, 1985). Root cause of major chronic diseases.
Lipid Peroxidation (LPO)	Chain reaction oxidative deterioration of PUFAs by free radicals — Initiation (•OH abstracts H) → Propagation (L• + O_2 → LOO• → LOOH → L•) → Termination.
MDA (Malondialdehyde)	Dialdehyde end-product of arachidonic acid peroxidation; most commonly measured LPO biomarker (TBARS assay); forms mutagenic DNA adducts (M2dG).
4-HNE (4-Hydroxynonenal)	Most cytotoxic aldehyde product of linoleic acid peroxidation; forms protein adducts (Michael addition with Cys, His, Lys); inhibits mitochondrial ETC.
F_2-Isoprostanes	Gold standard biomarker of lipid peroxidation in vivo; formed non-enzymatically from arachidonic acid by free radicals; measured in urine (8-iso-PGF2α).
Protein Carbonylation	Introduction of carbonyl groups (-C=O) into protein side chains by ROS; primary biomarker of protein oxidative damage; affects Pro, Arg, Lys, Thr residues; measured by DNPH assay.

8-OHdG	8-Hydroxy-2'-deoxyguanosine — primary product of •OH attack on guanine in DNA; gold standard biomarker of oxidative DNA damage; causes G→T transversion mutations; measured in urine.

Glycation	Non-enzymatic reaction of reducing sugars with free amino groups of proteins: Schiff base → Amadori product → AGEs. Accelerated in hyperglycaemia.

AGEs (Advanced Glycation End Products)	Irreversible carbonyl-containing cross-links formed from glycation and glycoxidation of proteins; bind RAGE receptor → NF-κB → chronic inflammation → diabetic complications.

Glycoxidation	Combined glycation + oxidative stress → rapid AGE formation via reactive dicarbonyls (methylglyoxal, glyoxal); explains accelerated tissue damage in uncontrolled diabetes.

Dietary Fibre	Edible plant components resistant to human digestive enzymes; fermented by colonic microbiota; produces SCFA; classified as soluble (cholesterol ↓, glycemia ↓) or insoluble (bulk, transit ↑).

Resistant Starch (RS)	Fraction of starch escaping small intestinal digestion; reaches large intestine intact; fermented to butyrate; Types RS1-RS5 based on resistance mechanism; functional as prebiotic.

Short Chain Fatty Acids (SCFA)	Metabolic products of colonic fermentation of dietary fibre — primarily acetate (C2), propionate (C3), and butyrate (C4). Butyrate = colonocyte energy source + anticancer agent.

Peroxynitrite ($ONOO^-$)	Non-radical RNS (Reactive Nitrogen Species) formed from NO• + $O_2•^-$; nitrates tyrosine residues → 3-nitrotyrosine; damages DNA and proteins; marker of nitrosative stress.

QUESTION BANK — 2 MARK QUESTIONS (Model Answers)

Q. Q1. Define Free Radical. Give two examples.

Ans: A free radical is any atom, molecule, or molecular fragment that has one or more UNPAIRED ELECTRONS in its outer orbital. The unpaired electron makes it paramagnetic and highly reactive. Denoted by a dot (•) superscript. Examples: (1) Hydroxyl radical (•OH) — most reactive biological radical; formed by Fenton reaction; attacks DNA, lipids, proteins; half-life in nanoseconds. (2) Superoxide anion radical ($O_2^{\bullet -}$) — primary ROS produced in cells by mitochondrial ETC Complex I and III; scavenged by Superoxide Dismutase (SOD).

Q. Q2. Write the Fenton reaction and Haber-Weiss reaction. What is the significance of each?

Ans: FENTON REACTION: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$. Significance: Iron ($Fe^{2+}$) catalyses conversion of H_2O_2 to the extremely reactive •OH. This explains why iron overload (haemochromatosis, haemosiderosis) causes tissue damage — excess Fe^{2+} drives Fenton reaction. HABER-WEISS REACTION (net): $O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + {}^{\bullet}OH + OH^-$. Significance: Net reaction combining Fenton + Fe^{3+} reduction by $O_2^{\bullet -}$ back to Fe^{2+}. Shows how two relatively mild ROS ($O_2^{\bullet -}$ and H_2O_2) combine (catalysed by trace iron) to produce the extremely damaging •OH.

Q. Q3. What is lipid peroxidation? Name the three phases.

Ans: Lipid Peroxidation (LPO) is the oxidative chain-reaction deterioration of polyunsaturated fatty acids (PUFAs) by free radicals, producing toxic aldehyde products and damaging biological membranes. It occurs in three phases: (1) INITIATION: A radical (•OH) abstracts a hydrogen atom from a bis-allylic C-H bond of PUFA → L• (carbon-centred radical) + H_2O; (2) PROPAGATION: L• + O_2 → LOO• (peroxyl radical); LOO• + LH → LOOH (lipid hydroperoxide) + L• → new round. Self-perpetuating — one initiating radical damages THOUSANDS of PUFAs; (3) TERMINATION: Two radicals react (LOO• + LOO• → products + O_2) OR chain-breaking antioxidant (Vitamin E) donates H to LOO• → LOOH + Toc•.

Q. Q4. What is 8-OHdG? Why is it called the 'gold standard' biomarker of oxidative DNA damage?

Ans: 8-OHdG (8-Hydroxy-2'-deoxyguanosine) is the oxidative modification product of guanine in DNA, formed when •OH attacks position C-8 of guanine. It is called the gold standard biomarker of oxidative DNA damage because: (1) Specific — unique product of •OH attack on guanine (most susceptible base); (2) Measurable — can be accurately measured in urine (repair excretion product), leukocytes, or tissue by HPLC-ECD or ELISA; (3) Clinically validated — elevated in cancer, diabetes, cardiovascular disease, smoking; (4) Reflects BOTH damage AND repair rate; (5)

Mutagenic — causes G→T transversion mutations (8-OHdG pairs with Adenine instead of Cytosine).

Q. Q5. Define Dietary Fibre. Differentiate between soluble and insoluble fibre with examples.

Ans: Dietary Fibre: Edible parts of plants resistant to human digestive enzymes, partially or completely fermented by colonic microbiota. (AACC/Codex Alimentarius). SOLUBLE FIBRE: Dissolves in water → forms viscous gel. Examples: Pectin (apple, citrus), Beta-glucan (oats, barley), Guar gum, Psyllium mucilage, Inulin/FOS. Functions: LDL cholesterol ↓ (bile acid sequestration), postprandial glucose ↓ (viscous gel slows absorption), prebiotic (fermented → SCFA). INSOLUBLE FIBRE: Does NOT dissolve in water. Examples: Cellulose, Lignin, Arabinoxylan (wheat bran). Functions: Increases stool bulk → speeds colonic transit → prevents constipation; reduces colorectal cancer risk; not significantly fermented.

Q. Q6. What is Resistant Starch? Give two types with examples.

Ans: Resistant Starch (RS) is the fraction of dietary starch that resists digestion in the small intestine and reaches the large intestine intact, where it is fermented by gut bacteria to produce SCFA (especially butyrate). Two important types: (1) RS2 — Resistant starch granules with B-type crystallinity (raw potato, unripe green banana, raw corn) — compact crystalline structure resists gelatinisation and amylase; destroyed by cooking; (2) RS3 — Retrograded starch formed when cooked starch is cooled (cooked-then-cooled potato, cold pasta salad, sushi rice) — linear amylose recrystallises on cooling forming resistant conformation; cooling potato increases RS3 content 3–4-fold compared to hot potato.

Q. Q7. Name the three primary enzymatic antioxidants. Write the reaction for each.

Ans: (1) Superoxide Dismutase (SOD): $2\ O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$. Locations: Cu/Zn-SOD (cytosol), Mn-SOD (mitochondria), EC-SOD (extracellular). Converts $O_2^{\bullet -} \rightarrow H_2O_2$. (2) Catalase (CAT): $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$. Location: Peroxisomes (mainly). Converts $H_2O_2 \rightarrow$ water + O_2 (very fast — fastest enzyme after SOD for its substrate). (3) Glutathione Peroxidase (GPx): $H_2O_2 + 2$ GSH $\rightarrow$ GSSG + $2\ H_2O$; also LOOH + 2 GSH → LOH + GSSG + H_2O. Cofactor: Selenium (Se) — GPx1 is a selenoprotein. Location: Cytosol and mitochondria. Works together with Glutathione Reductase (GSSG + NADPH → 2 GSH + $NADP^+$) to maintain GSH levels.

Q. Q8. What are AGEs? Name two diseases caused by AGE accumulation.

Ans: AGEs (Advanced Glycation End Products) are irreversible, stable end-products formed by the non-enzymatic reaction of reducing sugars with protein amino groups, followed by complex dehydration, oxidation, and condensation reactions (Maillard reaction in vivo). Examples: CML (Nε-Carboxymethyllysine), Pentosidine, GOLD. AGEs bind RAGE receptors → NF-κB activation →

chronic inflammation. Two diseases caused by AGE accumulation: (1) DIABETIC NEPHROPATHY — AGEs cross-link glomerular basement membrane proteins → thickening, altered permeability → proteinuria → progressive renal failure; (2) DIABETIC RETINOPATHY — AGEs stimulate VEGF (via RAGE/NF-κB) → neovascularisation → microaneurysms, exudates → leading cause of blindness in working-age adults.

QUESTION BANK — 5 MARK QUESTIONS

Q1. Describe the endogenous sources of free radical production in cells. (5 marks)

Q2. Explain the mechanism of free radical damage to lipids. Name three important products of lipid peroxidation with their biomarker significance. (5 marks)

Q3. Describe the free radical damage to nucleic acids. Explain the significance of 8-OHdG. (5 marks)

Q4. Classify dietary fibres. Describe the functional significance of any TWO soluble fibres. (5 marks)

Q5. What is Glycation and Glycoxidation? Describe the formation and clinical significance of AGEs. (5 marks)

QUESTION BANK — 10 MARK QUESTIONS

Q1. Write a comprehensive account of free radicals — definition, classification, production in cells, and their damaging reactions on Lipids and Proteins. (10 marks)

Q2. Define free radicals and ROS. Explain their damaging reactions on Carbohydrates and Nucleic Acids with clinical implications. (10 marks)

Q3. Write a detailed note on Dietary Fibres and Complex Carbohydrates as functional food ingredients — classification, mechanism, sources, and health benefits. (10 marks)

PREVIOUS-YEAR STYLE QUESTIONS

#	Question	Marks	Section
1	What are free radicals? Classify Reactive Oxygen Species (ROS) with examples and their sources.	5	Sections 1 & 2
2	Explain the Fenton reaction and Haber-Weiss reaction. What is the role of iron in free radical generation?	2-5	Section 2.2
3	Describe in detail the mechanism of lipid peroxidation. Name three biomarkers and their significance.	10	Section 4
4	Explain free radical damage to proteins. Name the types of protein oxidation and their biomarkers.	5	Section 5
5	Write a note on glycation and Advanced Glycation End Products (AGEs). Explain their role in diabetic complications.	10	Section 6
6	Define 8-OHdG. Explain its formation, mutagenic mechanism, and clinical significance as a biomarker.	5	Section 7.2
7	Classify dietary fibres with examples. Describe the health benefits of soluble dietary fibres.	10	Section 8

TOP 15 MCQs — WITH ANSWERS & EXPLANATIONS

Q1. Which of the following is the MOST REACTIVE free radical in biological systems?

(A) Superoxide anion radical ($O_2^{\bullet -}$)

(B) Hydroxyl radical (•OH)

(C) Peroxyl radical (ROO•)

(D) Nitric oxide radical (NO•)

✓ Correct: (B) Hydroxyl radical (•OH)

Explanation: •OH (Hydroxyl radical) is the MOST REACTIVE biological free radical. It has an extremely short half-life (nanoseconds) and attacks all biological molecules indiscriminately — DNA, lipids, proteins, carbohydrates. Unlike $O_2^{\bullet -}$ (which is mild and selective), •OH reacts at near diffusion-limited rates with ANY molecule it encounters. There is no specific enzyme scavenger for •OH.

Q2. The Fenton reaction involves which metal ion as the catalyst?

(A) Copper (Cu^{2+})

(B) Zinc (Zn^{2+})

(C) Iron (Fe^{2+})

(D) Manganese (Mn^{2+})

✓ Correct: (C) Iron (Fe^{2+})

Explanation: The Fenton Reaction: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {\bullet}OH + OH^-$. Fe^{2+} (ferrous iron) is the essential catalyst. Fe^{3+} is then reduced back to Fe^{2+} by $O_2{\bullet}^-$ (Haber-Weiss reaction), completing the catalytic cycle. This is why iron overload conditions (haemochromatosis, transfusion siderosis) cause severe tissue damage — excess Fe^{2+} perpetuates $\bullet OH$ generation. Copper can also catalyse a similar reaction (Fenton-like) but iron is the classical Fenton catalyst.

Q3. The PRIMARY site of superoxide radical ($O_2{\bullet}^-$) production in cells is:

(A) Lysosome

(B) Nucleus

(C) Peroxisome

(D) Mitochondrial electron transport chain (Complex I and III)

✓ Correct: (D) Mitochondrial electron transport chain (Complex I and III)

Explanation: The mitochondrial ETC — specifically Complex I (NADH dehydrogenase) and Complex III (cytochrome bc1 complex) — is the PRIMARY source of cellular $O_2{\bullet}^-$, contributing 70–90% of all cellular ROS. Electrons 'leak' from the electron transport chain and directly reduce O_2 to $O_2{\bullet}^-$. Complex I releases $O_2{\bullet}^-$ into the MATRIX; Complex III releases it into both the matrix and the intermembrane space.

Q4. In lipid peroxidation, Vitamin E terminates the chain reaction by:

(A) Chelating iron (Fe^{2+}) to prevent Fenton reaction

(B) Donating a hydrogen atom to peroxyl radical (LOO•) forming a stable tocopheroxyl radical

(C) Activating Superoxide Dismutase (SOD)

(D) Directly scavenging superoxide anion ($O_2{\bullet}^-$)

✓ Correct: (B) Donating a hydrogen atom to peroxyl radical (LOO•) forming a stable tocopheroxyl radical

Explanation: Vitamin E (α-Tocopherol) is the primary CHAIN-BREAKING antioxidant in lipid membranes. It donates a hydrogen atom (H•) from its phenolic -OH group to the

peroxyl radical (LOO•): LOO• + Toc-H → LOOH + Toc•. The resulting tocopheroxyl radical (Toc•) is stable and non-reactive — it does NOT propagate the chain. Toc• is then regenerated by Vitamin C: Toc• + Vitamin C → Toc-H + Asc•. This explains the 'Vitamin C spares Vitamin E' relationship.

Q5. The GOLD STANDARD biomarker for lipid peroxidation in vivo is:

(A) MDA (measured by TBARS assay)

(B) 4-HNE (4-Hydroxynonenal)

(C) F_2-Isoprostanes (8-iso-PGF2α in urine)

(D) Conjugated dienes

✓ Correct: (C) F_2-Isoprostanes (8-iso-PGF2α in urine)

Explanation: F_2-Isoprostanes (specifically urinary 8-iso-PGF2α) are the GOLD STANDARD biomarker of in vivo lipid peroxidation because they are: (1) Specific to free radical-mediated arachidonic acid peroxidation; (2) Stable compounds (unlike MDA); (3) Not affected by dietary fat intake; (4) Accurately measurable in urine, plasma, and tissue by GC-MS or ELISA. MDA (TBARS assay) is most commonly USED in research but is less specific. 4-HNE is the most CYTOTOXIC product.

Q6. Protein carbonylation is measured using which assay?

(A) TBARS assay

(B) DNPH (2,4-Dinitrophenylhydrazine) assay

(C) Griess reagent assay

(D) Ellman's reagent (DTNB) assay

✓ Correct: (B) DNPH (2,4-Dinitrophenylhydrazine) assay

Explanation: Protein Carbonyls are measured by the DNPH (2,4-Dinitrophenylhydrazine) assay: carbonyl groups (-CHO, C=O) react with DNPH → dinitrophenylhydrazone (DNP-hydrazone) → detected spectrophotometrically at 370 nm. This is the PRIMARY biomarker assay for oxidative protein damage. TBARS = lipid peroxidation (MDA). Griess = nitric oxide/nitrite. Ellman's (DTNB) = thiol groups (-SH, measuring GSH or free Cys).

Q7. 8-OHdG (8-hydroxy-2'-deoxyguanosine) causes which specific mutation?

(A) C → T transition

(B) A → G transition

(C) G → T transversion

(D) T → A transversion

✓ Correct: (C) G → T transversion

Explanation: 8-OHdG causes G → T TRANSVERSION (not transition). Mechanism: 8-OHdG can adopt a syn conformation and mispairs with ADENINE (instead of Cytosine). At the next round of replication: the incorporated Adenine pairs with Thymine → original G:C → mutant T:A. Transversion = purine (G) → pyrimidine (T). This G→T transversion is the most common mutation found in human cancers and specifically found in codon 12 of K-RAS in tobacco-associated lung cancer.

Q8. HbA1c (Glycosylated Haemoglobin) is correctly classified as:

(A) Advanced Glycation End Product (AGE)

(B) Schiff Base (aldimine)

(C) Amadori Product (ketoamine)

(D) Maillard product

✓ Correct: (C) Amadori Product (ketoamine)

Explanation: HbA1c is an AMADORI PRODUCT — specifically the Amadori rearrangement product of glucose + valine at the N-terminus of haemoglobin β-chain. Glycation sequence: Glucose + Hb → Schiff base (unstable, hours) → Amadori rearrangement → HbA1c (stable ketoamine, reflects ~3-month glucose). HbA1c is NOT an AGE (AGEs take months-years to form on long-lived proteins like collagen). This is the most commonly misanswered distinction in glycation questions.

Q9. Which dietary fibre has an FDA-approved health claim for reducing coronary heart disease risk?

(A) Psyllium husk

(B) Pectin

(C) Oat Beta-glucan

(D) Guar gum

✓ Correct: (C) Oat Beta-glucan

Explanation: OAT BETA-GLUCAN has the FDA-approved health claim (1997): 'Diets that include soluble fibre from oat bran or rolled oats and are low in saturated fat and cholesterol may reduce the risk of heart disease.' The minimum effective dose is 3g of oat β-glucan per day. This is also approved by EFSA (European Food Safety Authority). Psyllium also has an FDA qualified health claim for cholesterol reduction, but oat β-glucan was the FIRST and most recognised.

Q10. Resistant Starch Type 3 (RS3) is formed by:

(A) Physical entrapment of starch in intact cell walls

(B) B-type crystallinity of raw starch granules

(C) Retrogradation of amylose on cooling cooked starch

(D) Chemical cross-linking of starch chains

✓ Correct: (C) Retrogradation of amylose on cooling cooked starch

Explanation: RS3 (Retrograded starch) is formed when COOKED STARCH IS COOLED. During cooling, linear amylose molecules (which become mobile during gelatinisation in cooking) reassociate and re-crystallise into tight helical structures that resist amylase digestion. Classic examples: cooked-then-cooled potato (3–4× more RS than hot), cold pasta salad, sushi rice, cornflakes. Reheating partially converts RS3 back to digestible starch (RS3 content decreases on reheating).

Q11. The 'Respiratory Burst' or 'Oxidative Burst' in neutrophils is primarily mediated by:

(A) Xanthine Oxidase

(B) Cytochrome P450 CYP2E1

(C) NADPH Oxidase (NOX2)

(D) Monoamine Oxidase (MAO)

✓ Correct: (C) NADPH Oxidase (NOX2)

Explanation: NADPH Oxidase-2 (NOX2, also called gp91phox complex) mediates the respiratory burst in neutrophils and macrophages. Upon activation by pathogens: NADPH + $2O_2$ → $NADP^+ + H^+ + 2O_2^{\bullet-}$. O_2 consumption increases 10–20-fold. $O_2^{\bullet-}$ → H_2O_2 (by SOD) → HOCl (by Myeloperoxidase + Cl^-) → kills bacteria. Chronic Granulomatous Disease (CGD) = genetic NOX2 deficiency → inability to make $O_2^{\bullet-}$ → recurrent life-threatening bacterial/fungal infections.

Q12. Which enzyme is the primary antioxidant defence against lipid hydroperoxides in cell membranes?

(A) Superoxide Dismutase (SOD)

(B) Catalase

(C) Glutathione Peroxidase 4 (GPx4)

(D) Thioredoxin Reductase

✓ Correct: (C) Glutathione Peroxidase 4 (GPx4)

Explanation: GPx4 (Glutathione Peroxidase 4, also called Phospholipid Hydroperoxide Glutathione Peroxidase — PHGPx) is the ONLY enzyme that can directly reduce phospholipid hydroperoxides (LOOH) in cell membranes — without requiring them to be released from the membrane. All other GPx isoforms only work on free (non-esterified) hydroperoxides. GPx4 inhibition → uncontrolled lipid peroxidation → FERROPTOSIS (iron-dependent regulated cell death). GPx4 requires Selenium (Se) as a cofactor.

Q13. Peroxynitrite ($ONOO^-$) is formed from the reaction of:

(A) $H_2O_2 + Fe^{2+}$

(B) $O_2^{\bullet -} + NO^{\bullet}$

(C) $HOCl + H_2O_2$

(D) $O_2 + NADPH$

✓ Correct: (B) $O_2^{\bullet -} + NO^{\bullet}$

Explanation: Peroxynitrite ($ONOO^-$) is formed by the near-diffusion-limited reaction between superoxide ($O_2^{\bullet -}$) and nitric oxide ($NO^{\bullet}$): $O_2^{\bullet -} + NO^{\bullet} \rightarrow ONOO^-$ (rate constant $k \approx 10^{10}\ M^{-1}s^{-1}$ — faster than SOD scavenges $O_2^{\bullet -}$). This reaction is clinically important because it simultaneously: (1) removes the vasodilatory $NO^{\bullet}$ → endothelial dysfunction; (2) generates the highly toxic $ONOO^-$ which nitrates tyrosine residues (3-Nitrotyrosine) and causes DNA damage.

Q14. Which of the following dietary fibres has the HIGHEST viscosity and is used as a food thickener (E412)?

(A) Cellulose

(B) Psyllium

(C) Pectin

(D) Guar gum

✓ Correct: (D) Guar gum

Explanation: Guar gum (galactomannan from Cyamopsis tetragonoloba) has the HIGHEST viscosity of all natural gums and plant-derived fibres — approximately 8× more viscous than cornstarch at similar concentrations. It is used as a food additive E412 (thickener, stabiliser) in dairy products, sauces, bakery, and ice cream. Its high viscosity also explains its superior glycemic control effect among dietary fibres — viscosity directly correlates with slowing of glucose absorption.

Q15. LIGNIN is chemically classified as:

(A) A polysaccharide (glucose polymer)

(B) A glycoprotein

(C) A phenylpropanoid polymer (NOT a carbohydrate)

(D) A flavonoid

✓ Correct: (C) A phenylpropanoid polymer (NOT a carbohydrate)

Explanation: LIGNIN is NOT a carbohydrate — it is a complex aromatic PHENYLPROPANOID POLYMER composed of three monolignol monomers: p-Coumaryl alcohol (H), Coniferyl alcohol (G-guaiacyl), and Sinapyl alcohol (S-syringyl). It is classified as dietary fibre because it resists human digestion, but chemically it contains no sugar units. Important distinction: LIGNIN (structural polymer) ≠ LIGNAN (polyphenolic phytoestrogen dimers, e.g., SDG from flaxseed). This is one of the most common exam 'trap' questions.