Oxygen is an element indispensable for life. When cells use oxygen to generate energy, free radicals are created as a consequence of ATP (adenosine triphosphate) production by the mitochondria. These by-products are generally reactive oxygen species (ROS) that result from the cellular redox process. These species play a dual crucial role in human physiological and pathophysiological processes\(^1\). All ROS types, including superoxide anions and hydrogen peroxide, have unpaired valence electrons or unstable bonds. At high concentrations, ROS react readily with proteins, lipids, carbohydrates, and nucleic acids, often inducing irreversible functional alterations or even complete destruction. When ROS were initially integrated into biomedical concepts it was thought that they caused exclusively toxic effects and were associated with pathologies. ROS were once thought to originate almost entirely from mitochondrial metabolism. Indeed, while with normal mitochondrial function, the final oxygen electron receptor is reduced to water, it is possible, in particular under pathological conditions, that electrons leak out of the system prematurely and create ROS. However, there is mounting evidence that cellular enzymes referred to as NADPH oxidases are an important source of ROS in humans\(^2\). ROS-generating processes, including mitochondrial and NADPH oxidases, have a long and complex involvement in cellular and organismal function.
evolution and it is possible that ROS took part in early cell-to-cell signalling in unicellular organisms. It is now clear that organisms have also developed methods of utilising ROS in critical physiological processes. At the cellular level, ROS regulate growth, apoptosis and other signalling. At the systems level they contribute to complex functions such as blood pressure regulation, cognitive function and immune function. ROS enable the response to growth factor stimulation and the generation of the inflammatory response, as well as having vital roles in the immune system where they directly kill pathogens. Other examples of a biochemical role of ROS are found in primitive organisms, where ROS are involved in the cross-linking of the extracellular matrix and in the hardening of the fertilization envelope after egg-sperm fusion. However, there is a delicate balance between appropriate redox states and oxidative stress, dependent on the relative rates of production and degradation. For specificity in signalling, biological systems recognize the amount and type of ROS, as well as the temporal and spatial distribution. Overproduction of ROS occurs during stimulated disease conditions, but there is also a genetic component to the propensity for ROS generation. Natural human antioxidant defenses are not always sufficient to maintain the proper ROS balance. Also, a normal process can become pathological when it persists longer. The mechanisms of senescence involve a contribution from free radicals, leading to the proposition more than fifty years ago that the aging process results partly from oxidative damage. Oxidative stress is associated with the general aging process and cell death, affecting all major organ systems and ROS have a part in many age-associated diseases, including Alzheimer’s disease, Parkinson’s disease and virtually all cardiovascular diseases. Large epidemiological studies support the relationship between oxidative state and global health; high consumption of foods rich in antioxidants is associated with lower disease rates and preventive protection.

**Biochemistry of reactive oxygen species:**

Most of the oxygen taken up by the cells of our body is converted into H₂O during mitochondrial respiration. However, a small percentage of oxygen (less than 5%) is converted into ROS. ROS can be neutral molecules (hydrogen peroxide), ions (superoxide anion) or radicals (hydroxyl radicals). Due to their reactivity, ROS are observed as a cascade of transitions from one species to another (Fig. 1). These substances are highly toxic in nature and if allowed to accumulate, they can destroy all the macromolecules of the cells like lipids, proteins, mitochondrial and nuclear DNA molecules.

When oxygen accepts a single electron, it forms a superoxide radical. Superoxide does not readily cross membranes and is short-lived and local in its effect, once superoxide radical is formed, it quickly undergoes dismutation to form longer-lasting and membrane-diffusible H₂O₂ by the family of enzymes known as superoxide dismutase (SOD). Finally, H₂O₂ is converted into a highly reactive hydroxyl radical (OH) in the presence of transition metal ions like Cu²⁺, iron (Fe⁴⁺) through Fenton reaction or Haber-Weiss reaction. H₂O₂ is normally disposed as H₂O and O₂ by two important antioxidant enzymes like glutathione peroxidase and catalase. The hydroxyl radical that are formed through Fenton reaction are highly destructive in nature and can destroy lipid, proteins and DNA molecules of the cells. It also removes H⁺ ions from the polyunsaturated fatty acid (PUFA) molecules of the cell membrane. Thus, lipid peroxidation induced by OH radicals will become self-propagating in nature that can eventually lead to destruction of all lipids in the cell membrane.

In order to understand the chemical properties and reactivity’s of each species are listed in the Table 1. The reactivity reaction rate is based on the reactions that these species abstract doubly allylic hydrogen of highly unsaturated fatty acids such as linoleic acid or arachidonic acid, assuming the rate of peroxyl radical as one. It is clear that OH has the

<table>
<thead>
<tr>
<th>Table 1 : Relative activities of ROS</th>
<th>Symbol</th>
<th>Half-life (seconds)</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide</td>
<td>O₂⁻</td>
<td>10⁻⁶ sec</td>
<td>0</td>
</tr>
<tr>
<td>Hydroxyl radical</td>
<td>HO⁻</td>
<td>10⁻⁹ sec</td>
<td>10⁷</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>H₂O₂</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Peroxyl radical</td>
<td>ROO⁻</td>
<td>Few sec</td>
<td>1</td>
</tr>
<tr>
<td>Organic hydroperoxide</td>
<td>ROOH</td>
<td>Stable</td>
<td>*</td>
</tr>
<tr>
<td>Singlet oxygen</td>
<td>O₂⁻</td>
<td>10⁻⁶ sec</td>
<td>0</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>NO⁻</td>
<td>Few sec</td>
<td>*</td>
</tr>
<tr>
<td>Peroxynitrite</td>
<td>ONOO⁻</td>
<td>10⁻³ sec</td>
<td>*</td>
</tr>
<tr>
<td>Nitrogen dioxide</td>
<td>NO₂⁻</td>
<td>Few sec</td>
<td>*</td>
</tr>
</tbody>
</table>

Relative activities of ROS are expressed as a ratio of the rate constant between peroxyl radical and lipid. * Represent the nonability of data under the same condition.
highest reaction rate as rapid as a diffusion limited rate (that it reacts with the first molecule, it comes into contact with). Singlet oxygen is also reactive but the reaction rate with lipids is not as high as .OH \[^{14}\].

**Sources of ROS:**

Many systems (enzymatic and non enzymatic) can be considered as sources of ROS in the living organism\[^{15,16,17}\]. Exogenously, ROS are produced from exposure to environmental agents such as ultra violet (UV) radiation and redox-cycling agents\[^{18}\]. Endogenously, ROS are derived mostly from the incomplete reaction of oxygen during aerobic metabolism in vivo. They are produced from mitochondrial electron transport chain, NADH/NADPH oxidases, arachidonic acid pathway enzymes, cyclooxygenase and lipoxygenase, xanthine oxidases, phagocytes - derived myeloperoxidase \[^{19,20,21}\]. There is strong evidence showing that the principal source of superoxide anion in the vascular system prevails in the NADPH oxidase metabolism\[^{22}\]. Nitric oxide synthase (NOS) is the enzyme primarily responsible for the production of nitric oxide (NO). Nitric oxide synthase has been reported to generate superoxide anion in condition of substrate (arginine) or cofactor (BH4) depletion\[^{23,24}\]. This condition has introduced the concept of “NOS uncoupling” whereby NOS favoured superoxide anion formation over nitric oxide production\[^{24}\]. Uncoupled NOS, is a dysfunctional endothelium NOS which promote an excessive release of superoxide anion and hydrogen peroxide subsequent to peroxynitrite-mediated cellular injury\[^{24,25,26}\]. Xanthine oxidoreductase (XOR) is a complex molybdoflavoenzyme that is readily available from mammalian or human milk\[^{27,28}\]. Several studies reported that inhibition of XOR has been found to normalize the overproduction of superoxide anion which confirms XOR effects on the surplus production of ROS in vascular cells (Fig. 2).

The generation of mitochondrial ROS is a consequence of oxidative phosphorylation. At several sites along the cytochrome chain, electrons derived from NADH or FADH can directly react with oxygen or other electron acceptors and generate free radicals. It has recently been proposed that mitochondrial ROS play a crucial role in several redox-dependent signalling

---

**Fig. 2: Reactive oxygen species pathway in health and disease**

- Physicochemical agent
- NADH/NADPH Oxidase
- Endothelial uncoupled NOS
- UV radiation
- Xanthine oxidase
- Physiology pathway
  - Hydrogen peroxide \([H_2O_2]\)
  - Catalase, GPx
  - Fenton reaction \(H_2O + O_2 \rightarrow OH\)
- Pathological pathway
  - Peroxynitrite
  - Peroxynitrite decomposition
  - DNA damage, protein nitrosilition
  - Lipid peroxidation
  - Cell damage, Cancer, DEATH
  - Cell damage, Atherosclerosis, CVD, inflammation, DEATH

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processes as well as in the aging clock (Fig. 3).

Production of NO:
The enzyme NOS exists in three isoforms, i.e., neuronal NOS (nNOS; type I)[29], inducible NOS (iNOS; type II)[30] and endothelial NOS (eNOS; type III) [31]. Many tissues express one or more of these isoforms. The isoforms nNOS and eNOS are constitutively expressed, but their activity is regulated by the intracellular calcium concentration. The isoform iNOS is inducibly expressed in macrophages after stimulation by cytokines, lipopolysaccharides and other immunologically relevant agents[32]. Expression of iNOS is regulated at the transcriptional and posttranscriptional level by signaling pathways that involve agents such as the redox-responsive transcription factor NF-kB or mitogen-activated protein kinases (MAPKs)[33]. The rate of NO synthesis is also determined to some extent by the availability of the substrate L-arginine and by the cofactor tetrahydrobiopterin (BH4).

ROS production by phagocytic NADPH oxidase (Oxidative burst):
Activated macrophages and neutrophils can produce large amounts of superoxide and its derivatives via the phagocytic isoform of NADPH oxidase. This enzyme is a heme-containing protein complex illustrated schematically in Fig. 5. In an inflammatory environment hydrogen peroxide is produced by activated macrophages at an estimated rate of 2–6x10^{-14} mol.h^{-1}.cell^{-1} and may reach a concentration of 10–100µM in the vicinity of these cells[34-36]. The massive production of antimicrobial and tumoricidal ROS in an inflammatory environment is called the “oxidative burst” and plays an important role as a first line of defense against environmental pathogens. The physiological relevance of NADPH oxidase as a defense agent is suggested by the observation that mice lacking the NADPH oxidase components gp91phox or p47 exhibit reduced resistance to infection[37-39]. The combined activities of NADPH oxidase and myeloperoxidase in phagocytes leads, in addition, to the production of hypochlorous acid (HClO), one of the strongest physiological oxidants and a powerful antimicrobial agent[34,40]. Stimulated neutrophils and macrophages generate also singlet oxygen by reactions that involve either myeloperoxidase or NADPH oxidase[41]. Importantly, however, physiologically relevant ROS concentrations can

---

**Fig. 3**: Sources of ROS and their cellular oxidative interactons[34]
also modulate redox-sensitive signal cascades and enhance immunological functions of lymphocytes. Phagocytic NADPH oxidase becomes activated upon translocation of cytosolic p47, p67 and a G protein of the rac family to the membrane-bound cytochrome b558 complex that contains gp91phox and p22 (Fig. 4). The catalytic moiety gp91phox is a plasma membrane-associated complex protein containing a flavin-adenine dinucleotide component and two hemes. The activation of phagocytic NADPH oxidase can be induced by microbial products such as bacterial lipopolysaccharide, by lipoproteins or by cytokines such as interferon-g, interleukin-1b, or interleukin-8. The activation of NADPH oxidase is mainly controlled by the rac isoform rac2 in neutrophils and rac1 in macrophages.

**Natural defense system:**
Nature has endowed each cell with adequate protective mechanisms against any harmful effects of free radicals: superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, thioredoxin, thiols and disulfide bonding are buffering systems in every cell. α-Tocopherol (vitamin E) is an essential nutrient, which functions as a chain-breaking antioxidant and prevents the propagation of free radical reactions in all cell membranes in the human body. Ascorbic acid (vitamin C) is also a part of the normal protecting mechanism. Other non-enzymatic antioxidants include carotenoids, flavonoids and related polyphenols, α-lipoic acid, glutathione etc. All these antioxidant, their functions along with their location are summarized in Table 2.

Antioxidants affect can be achieved in 3 different ways (Fig. 5) as: 1. Intracellular environment contains enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx),

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Function</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Enzymes and proteins)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. SOD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. CU-Zn SOD</td>
<td>Scavenge $O_2^-$</td>
<td>Erythrocyte cytoplasm</td>
</tr>
<tr>
<td>b. Mn-SOD</td>
<td>Scavenge $O_2^-$</td>
<td>Mitochondria</td>
</tr>
<tr>
<td>c. EC-SOD</td>
<td>Scavenge $O_2^-$</td>
<td>Blood, plasma, vessel wall</td>
</tr>
<tr>
<td>2. Catalase</td>
<td>Scavenge $H_2O_2$</td>
<td>Peroxisome</td>
</tr>
<tr>
<td>3. GPx</td>
<td>Degradation of $H_2O_2$ and lipid peroxide</td>
<td>Cytoplasm, mitochondria</td>
</tr>
<tr>
<td>4. GST</td>
<td>Degradation of $H_2O_2$ and lipid peroxide</td>
<td>Cell membrane, cytoplasm, Mitochondria, Endoplasmic reticulum</td>
</tr>
<tr>
<td>5. Ferritin</td>
<td>Fe$^{2+}$ Chelating</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>6. Transferrin</td>
<td>Fe$^{2+}$ Chelating</td>
<td>Extracellular Fluid</td>
</tr>
<tr>
<td>7. Lactoferrin</td>
<td>Fe$^{2+}$ Chelating</td>
<td>Extracellular Fluid</td>
</tr>
<tr>
<td>8. Ceruloplasmin</td>
<td>Cu$^{2+}$ Chelating, oxidation of Fe$^{2+}$. Scavenge $O_2^-$</td>
<td>Extracellular Fluid</td>
</tr>
<tr>
<td>9. Albumin</td>
<td>Cu$^{2+}$ Chelating, Scavenge OH, LOO., HOCl etc</td>
<td>Extracellular Fluid</td>
</tr>
</tbody>
</table>

| Low molecular compounds |                                                   |                         |
| 1. Vitamin E        | Scavenge OH, LOO., HOCI etc                       | Biomembrane             |
| 2. Ubiquinone       | Scavenge OH, LOO., HOCI etc                       | Biomembrane             |
| 3. Carotenoid       | Scavenge OH, LOO., HOCI $^1$O$_2$                 | Biomembrane             |
| 4. Vitamin C        | Scavenge OH, $O_2^-$                              | Cytoplasm               |
| 5. GSH              | Scavenge OH, $O_2^-$                              | Cytoplasm, Mitochondria |
| 6. Uric Acid        | Prevention of lipid peroxidation                  | Blood                   |
| 7. Bilirubin        | Prevention of lipid peroxidation                  | Blood                   |
glutathione-S-transferase (GST) and low molecular weight antioxidant glutathione (GSH) that catalyze the breakdown of oxidants generated by cellular metabolism. 2<sup>nd</sup> Preventive antioxidant proteins exist to sequester free transitional metal ions such iron and copper include the iron-binding protein transferring and copper-binding proteins ceruloplasmin and albumin. These antioxidants preventing their interaction with $\text{H}_2\text{O}_2$ and $\text{O}_2^-$ which would facilitate the production of the produce highly reactive hydroxyl radical ($\cdot\text{OH}$). 3<sup>rd</sup> chain breaking (sacrificial) antioxidants are powerful electron donors and react preferentially with free radicals before more important target molecules are damaged. In doing so, the antioxidant is sacrificed (oxidized) and must be regenerated or replaced (Fig.6). By, definition, the antioxidant radical is relatively unreactive and unable to attack further molecules<sup>[46-48]</sup>.

**Physiological role of reactive oxygen species in health:**

Reactive oxygen species present a paradox in their biological function as summarized in Fig.12, on one hand; they prevent disease by assisting the immune system, mediating cell signaling and playing an essential role in

---

**Fig. 5:** Antioxidant defenses against free radical attack: Antioxidant enzymes catalyze the breakdown of free radical species, usually in the intracellular environment. Transition metal binding proteins prevent the interaction of transition metals such as iron and copper with hydrogen peroxide and superoxide producing highly reactive hydroxyl radicals. Chain breaking antioxidants are powerful electron donors and react preferentially with free radicals before important target molecules are damaged. In doing so, the antioxidant is oxidized and must be regenerated or replaced. By definition, the antioxidant radical is relatively unreactive and unable to attack further molecules.

**Fig. 6:** Role of vitamin E, vitamin C and glutathione in antioxidant network.
**Table 3: A summary of key signaling factors**

<table>
<thead>
<tr>
<th>Name</th>
<th>Symbol</th>
<th>Function</th>
<th>Effect on ROS/Oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoptosis signal-regulating kinase 1</td>
<td>ASK1</td>
<td>ASK1 activates of JNK and P38 and is inactive when complexed with Trx</td>
<td>Causes dissociation of the Trx–ASK1 complex leading to ASK1 activation of JNK and P38</td>
</tr>
<tr>
<td>Caspase 8</td>
<td></td>
<td>A family of cytosolic aspartate-specific cysteine proteases involved in the initiation and execution of apoptosis; they are expressed as latent zymogens and are activated by an autoproteolytic mechanism or by processing by other proteases (frequently other caspasas)</td>
<td>Activated by ROS</td>
</tr>
<tr>
<td>Epidermal growth factor receptor</td>
<td>EGFR</td>
<td>A member of the EGFR family of receptor tyrosine kinases, which activates Raf and MAPKK leading to activated ERK</td>
<td>Activated by ROS</td>
</tr>
<tr>
<td>Extracellular signal-regulated kinases</td>
<td>ERK</td>
<td>Members of the MAPK family; regulates cell proliferation and triggers NADH oxidase; ERK1 and ERK2 (also known as MAPK3 and MAPK1) are part of the Ras-Raf-ERK signal transduction cascade often found downstream of growth factor receptor activation.</td>
<td>Activated by ROS</td>
</tr>
<tr>
<td>Hypoxia-inducible factor</td>
<td>HIF-1</td>
<td>Induces erythropoietin, VEGF and TH; the hypoxia-inducible transcription factor 1 alpha (HIF-1 alpha) is the regulated member of the hypoxia-inducible transcription factor heterodimer HIF-1 alpha; HIF-1 alpha binds to hypoxia-response elements in the promoters of many genes involved in adapting to an environment of insufficient oxygen or hypoxia.</td>
<td>Stimulated by low oxygen</td>
</tr>
<tr>
<td>Interleukin 1-beta</td>
<td>IL1β</td>
<td>Produced by a wide variety of cells in response to stimuli such as those produced by inflammatory agents, infections or microbial endotoxins</td>
<td>ROS increases activity</td>
</tr>
<tr>
<td>Interleukin 6, also known as interferon-beta</td>
<td>IL6</td>
<td>A multifunctional protein that plays important roles in host defense, acute phase reactions, immune responses and hematopoiesis.</td>
<td>ROS decreases activity</td>
</tr>
<tr>
<td>c-Jun N-terminal kinase</td>
<td>JNK</td>
<td>A member of the MAPK family; promotes phosphorylation and activation of AP-1 (c-Jun component); leads to apoptosis or necrosis activated by TNF-α; members of the MAPK family, the JNKs, are activated by environmental stresses and inflammatory cytokines.</td>
<td>ROS leads to activation following dissociation of the Trx-apoptosis signal-regulating kinase 1 or disruption of glutathione-s-transferase binding</td>
</tr>
<tr>
<td>Mitogen-activated protein kinase</td>
<td>MAPK</td>
<td>Activates ERK upon phosphorylation</td>
<td></td>
</tr>
<tr>
<td>Nicotinamide adenine dinucleotide oxidase</td>
<td>NADH</td>
<td>A potential source for O2 in the proliferation-inducing activity of oxLDL</td>
<td>Activated by ROS as Inβ (inhibitor) and is degraded by oxidation: NNFβ DNA-binding complex is inhibited by Inβ proteins, which inactivate NNFβ by trapping it in the cytoplasm is decreased</td>
</tr>
<tr>
<td>Nuclear factor of kappa light chain gene enhancer in B cells</td>
<td>NNFβ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P38</td>
<td></td>
<td>A member of the MAPK family: substrates include transcription regulators activating transcription factor 2 (ATF2), myocyte enhancing factor 2c (MEF2C) and MAX (a Myc heterodimerization partner), cell cycle regulator Cdc25B, and tumor suppressor p53, which suggest the roles of this kinase in stress related transcription and cell cycle regulation, as well as in genotoxic stress response</td>
<td>Activated by ROS</td>
</tr>
<tr>
<td>P53</td>
<td></td>
<td>Tumor suppressor protein is a multifunctional transcription factor that regulates cellular decisions regarding proliferation, cell cycle checkpoints and apoptosis</td>
<td>ROS Increases accumulation of protein levels decreases binding to DNA</td>
</tr>
<tr>
<td>Signal transducer and activator of transcription</td>
<td>STAT1 and 2</td>
<td>Inflammatory cytokine STAT proteins are a family of latent cytoplasmic transcription factors involved in cytokine, hormone and growth factor; signal transduction regulates numerous aspects of hematopoiesis and the immune response via activation of the JAK/ STAT pathway; the activated Stat proteins, in turn, dimerize, translocate into the nucleus and activate a specific set of genes; in this way, each cytokine elicits its specific response from the target cell</td>
<td>Activated by ROS</td>
</tr>
<tr>
<td>Vascular endothelial growth factor</td>
<td>VEGF</td>
<td>its ability to increase the permeability of capillary blood vessels</td>
<td>Decreased by ROS</td>
</tr>
</tbody>
</table>
apoptosis. On the other hand, they can damage important macromolecules in cells and may have a role in carcinogenesis and CVD. The generation of ROS historically was viewed as indiscriminate and random and their targets as primary determinants of disease and aging\cite{49-50}. Research also demonstrates that ROS generation is a normal physiological process, particularly for proper immunocompetence and in coordination and activation of numerous signal transduction pathways\cite{50}.

**Role of ROS in signal transduction and regulation of proliferation:**

Controlled production of ROS is essential for the activity of signal transduction pathways (key signaling factors are summary in Table 3) and one broad class of signal transduction molecules on which ROS influence function is the mitogen-activated protein kinases (MAPKs). MAPKs are composed of three subfamilies: the extracellular signal regulated kinases (ERKs); ERK1 and ERK2; the c-Jun N-terminal kinases (JNKs); JNK1, JNK2 and JNK3 and the p38 kinases; p38a, p38b, p38g and p38\cite{51,52}.

ERKs traditionally regulate cell proliferation; JNKs and p38 kinases are more strongly linked to stress responses ultimately leading to apoptosis or necrosis\cite{50}. ERKs coordinate signaling through growth factors such as platelet-derived growth factor (PDGF), fibroblast growth factor, (FGF) and epidermal growth factor (EGF)\cite{53,54}. Epidermal growth factor receptor (EGFR) has been demonstrated to be activated by ROS, similar to its activation by binding of its ligand, EGF\cite{50,55}. Activation of EGFR causes receptor autophosphorylation, recruitment of accessory proteins, and activation of membrane-localized “Ras”, which initiates phosphorylation and activation of “Raf” and MAPK kinases, ultimately activating ERK\cite{56}. Activation of ERKs by this route also triggers NADPH oxidase activity, producing superoxide anions and hydrogen peroxide\cite{52,57}. This transient increase in intracellular ROS can mimic or augment ligand–receptor binding by facilitating autophosphorylation of the receptors and induction of signaling cascades (Fig. 7); in fact, elimination of hydrogen peroxide by catalase has been demonstrated to inhibit EGF and nerve growth factor receptor autophosphorylation, as well as phosphorylation of downstream effector proteins.

Another means by which ROS potentiates and coordinates growth factor action through MAPKs is by the action of hydrogen peroxide on glutathione-sensitive, membranebound phosphatases that are necessary for dephosphorylation and, thus, down-regulating active growth factor receptors\cite{52}. These phosphatases contain redox-sensitive cysteine residues; increased levels of ROS can react with these residues, transiently inactivating the phosphatases\cite{49,58,59}. With kinase activity proceeding unopposed by phosphatase activity, the net result is an increase in phosphorylation levels and pathway activation.

Increasing awareness of the role of ROS as second messengers in diverse signaling pathways has lead researchers to speculate that this may allow a cooperativity or synergism of response from different extracellular stimuli\cite{51}. Ultimately, cellular response to oxidative stress and growth factors requires a balance between signals promoting cell proliferation or growth inhibition and/or cell death (Fig. 8).

**ERK activation generally promotes survival of cells in response to ROS**\cite{49} but also can promote apoptosis under
certain conditions and in certain cell types. For example, ERK facilitates hypoxia-induced apoptosis of macrophages and cisplatin-induced apoptosis of HeLa cells\[^50\]. Whether ERK promotes cell survival or cell death may be determined by the characteristics of ERK activation. Survival appears to be enhanced by rapid, transient activation of ERK, whereas apoptosis results from a slower, more sustained activation. This may reflect differences in cellular response to acute vs. chronic oxidative stress\[^50\]. The JNK and p38 families of MAPK sometimes are known collectively as the stress activated protein kinases (SAPKs) for their role in cellular response to different stresses, including cytokines, radiation, osmotic shock, mechanical injury, heat shock and oxidative damage\[^51\]. Activation of these kinases involves multiple MAPKs and interacting regulatory proteins. As JNK and p38 kinases are activated by a wide array of stresses. For example, the redox regulated protein thioredoxin (Trx) binds to and inhibits the activity of the apoptosis signal-regulating kinase 1 (ASK1) under normal redox conditions. Oxidative stress causes dissociation of the Trx–ASK1 complex, leading to activation of JNK and p38 by ASK1\[^51\]. Similarly, binding of glutathione-s-transferase to JNK under non-stressed conditions can be disrupted by oxidative stress, resulting in activated JNK\[^50,51\]. One important downstream effect of JNK activation is activation of the transcription factor AP-1. Oxidative activation of JNKS promotes phosphorylation of serine residues 63 and 73 of the aminoterminal transactivation domain of c-Jun, part of the dimeric AP-1 transcription factor and leading to transactivation of AP-1-regulated genes. JNKS have been observed to be activated in response to environmental stressors that also activate AP-1, such as radiation, UV light, and certain growth factors\[^52\]. Members of the p38 family are activated in immune cells by inflammatory cytokines as well as by stimuli such as hormones, G-protein-coupled receptor ligands and stresses such as osmotic shock and heat shock. Given their role in regulating expression of cytokines, p38 kinases are thought to be involved in the pathogenesis of diseases such as asthma and some autoimmune conditions\[^51,56\].

ROS coordinate and enhance activity of signal transduction cascades in response to extracellular stimuli, changes at the level of gene expression ultimately influences the decision of the cell to proliferate or undergo apoptosis. As mentioned above, JNKS promote phosphorylation and activation of the c-Jun component of the transcription factor AP-1, leading to up-regulated transcription of AP-1-regulated genes. In addition to AP-1, the transcription factor hypoxia-inducible factor 1 (HIF-1) provides another example of transcriptional regulation mediated by ROS. Activity of HIF-1 is controlled by changes in oxygen tension. Decreasing oxygen levels enhances formation of the active HIF-1 heterodimer complex, promoting transcription of HIF-1-inducible genes, such as those encoding erythropoietin, vascular endothelial growth factor (VEGF) and a tyrosine hydroxylase (TH) that participates in control of ventilation by the carotid body\[^52\]. This indicates that changing levels of ROS may be influenced by oxygen levels in the cell (Fig. 9). This may provide one way that cells could sense and adjust oxygen levels.

As another example, the transcription factor nuclear factor kappa B (NFkB), which participates in a wide variety of biological processes including inflammation, growth control and apoptosis, was the first eukaryotic transcription factor shown to respond directly to oxidative stress in some cell types\[^55,60,61\]. Although activity of this transcription factor is not strictly dependent on ROS, oxidative stress has been observed to be a strong activator of NFkB in some cell types. There are at least two ways by which NFkB activity is influenced by ROS levels. First, ROS enhance degradation of the NFkB inhibitor, Inß, resulting in increased levels of active NFkB in the nucleus and leading to increased NFkB DNA binding. Induction of Inß kinase a, which phosphorylates Inß, marking it for degradation by the proteasome, is observed in Jurkat cells after a moderate prooxidative shift in the intracellular redox state. ROS also can influence NFfB by direct action on the transcription factor itself. NFkB must be in a reduced form to bind DNA, and reducing agents such as dithiothreitol and hemcaptopoethanol have been observed to enhance NFkB DNA binding\[^62\]. Increased NFkB activity results in increased transcription of NFkB-regulated genes, such as tumor necrosis factor receptor-associated factor 1 and 2 and the cellular inhibitors of apoptosis proteins, which all have antiapoptotic properties\[^63,64\]. NFkB, however, also can activate proapoptotic genes such as “Fas” ligand and p53.
While some studies have shown both prosurvival and proapoptotic effects of NFnβ activation, most show a proapoptotic effect following oxidative injury. The exact mechanisms that is physiologically responsible for the redox balance in cells and the relationship with cell survival and apoptosis is still an area which will benefit from further investigation.

**Immunological functions of ROS:**

ROS have also been observed to have important roles in proper functioning of the innate immune response, activation of the adaptive immune response, as well as down regulation of inflammation and immune system activity. Disruption or dysregulation of immune system functions can lead to diseases characterized by inflammation, including atherosclerosis and cancer.

![Image of ROS functions in the immunological response against environmental pathogens.](image)

**Fig. 10:** Functions of ROS in the immunological response against environmental pathogens. The massive production of ROS (oxidative burst) by activated macrophages in the inflammatory environment provides a first line of defense against environmental pathogens. A certain fraction of pathogens, however, may escape this rapid but moderately effective manifestation of “innate immunity” and may generate within a few days a large progenesis of pathogens. Antigenic peptides generated within the activated macrophages by the breakdown of pathogens are presented by major histocompatibility complex (MHC) determinants to the antigen receptors (AR) of T lymphocytes. This interaction triggers the proliferation and differentiation of the T cells and leads within a few days to a large progenesis of immunological effector cells. The effector cells provide a highly effective and antigen-specific immunological defense. ROS that are concomitantly produced by the activated macrophages in the inflammatory environment enhance the AR-mediated signal cascades and decrease thereby the activation threshold of the T cells. Without this effect, the T lymphocytes would require relatively large concentrations of antigenic peptides and would lose valuable time in their “race” with the proliferating pathogens. In this situation time may be a matter of life or death for the organism.

During the initial response to an invading pathogen, activation of the innate immune response, characterized by generation of ROS within phagocytic cells such as macrophages and neutrophils, is a critical event in the initiation of phagocytosis and subsequent destruction of these microorganisms. In the production of high levels of superoxide anions and hydrogen peroxide by NADPH oxidase, the respiratory (or better, oxidative) burst is required for destruction of engulfed pathogens. Defects in any of a number of phagocyte-based NADPH oxidase enzymes has been shown to result in chronic granulomatous disease (CGD) which is characterized by increased susceptibility and mortality due to bacterial and fungal infections. Engulfment of pathogenic bacteria can trigger this oxidative burst, mediated in part by bacterial lipopolysaccharide (LPS) activation of the transmembrane Toll-like receptor 4 (TLR4), during the host innate immune response. Stimulation of TLR4 by LPS also induces signaling pathways that activate NFnβ and promote production of the inflammatory cytokine interleukin (IL) 8; both these activities can be blocked by antioxidants.

In 2004, Liu et al. suggests that the NADPH oxidase system is key in the generation of ROS following respiratory syncytial virus (RSV) infection. The authors also demonstrate that generation of ROS promotes activation of signal transduction pathways responsible for the production of inflammatory cytokines and chemokines, apparently by ROS-mediated inactivation of intracellular tyrosine phosphatases. This allows increased phosphorylation and activity of signal transducer and activator of transcription (STAT) 1 and 3, transcription factors that regulate expression of interferon regulatory factors 1 and 7. These regulatory factors, in turn, promote up-regulation of genes necessary for an effective antiviral response. In addition to ROS role in RSV infection, other groups have shown roles for virus-induced ROS activation in influenza, HIV, hepatitis B and hepatitis C infections.

The generation of ROS during the innate immune response also modulates apoptosis of neutrophils at the site of inflammation. Failure to down-regulate these processes can lead to pathologically chronic inflammation. Several groups have shown that a sustained and robust oxidative burst is required for neutrophil apoptosis. In 2003, Zhang et al. demonstrated that control of apoptosis by ROS is achieved by ROS promotion of cleavage and activation of caspase 8, which, in turn, activates caspase 3, triggering apoptosis. Pretreatment of neutrophils with an NADPH oxidase inhibitor blocked caspase 8 processing and subsequent apoptosis; similarly neutrophils from CGD patients deficient in NADPH oxidase activity fail to undergo apoptosis. ROS formation triggered by phagocytosis also inhibits the generally prosurvival ERK pathway. However, within the inflammatory environment, neutrophils are exposed to prosurvival growth factors such as granulocyte–macrophage colony stimulating factor (GM-CSF), which sustains activation of the ERK pathway. Conversely, GM-CSF can trigger an oxidative burst, down-regulating ERK.
This balanced crosstalk between proapoptotic and prosurvival signals allows neutrophils to survive long enough to complete necessary phagocytosis but eliminates the neutrophils once they have reached the end of their useful lifespan. In a similar situation, most activated antigen-specific T lymphocytes must die after the immune response peaks to prevent autoimmunity and ensure T-cell homeostasis. ROS involvement in activated T-cell apoptosis has been demonstrated by Hildeman et al., 1999 [73], who showed that T-cell death was characterized by superoxide generation and that a superoxide dismutase mimic could prevent T-cell apoptosis.

ROS also provide a connection between the innate immune response and subsequent adaptive immune responses, both by themselves and by their effects on production of proinflammatory cytokines. In the initial stages of infection, antigen levels often are suboptimal for activation of T cells through coordinated binding to the Tcell receptor CD3 and the costimulatory receptor CD28. However, survival of the host often is dependent on initiation of an immune response even before optimal antigen levels are attained. In 2000, Hehner et al.[76] demonstrated that exposure of T cells to macrophage-produced hydrogen peroxide or l-lactate facilitates a shift in the glutathione/glutathione disulfide ratio in the cell, allowing activation of response even at low antigen levels. Physiologically relevant amounts could substitute for antigen binding to CD3 but not to CD28. This ROS-induced shift in glutathione/glutathione disulfide ratio also was accompanied by activation of the MAPKs JNK and p38, signaling intermediates activated by ROS. Thus, production of ROS and their subsequent action on T cells may allow an early and robust immune response even at low levels of stimulating antigens. Additionally, up-regulation of inflammatory cytokine expression and production by ROS, as discussed above, is necessary for subsequent activation of T cells by macrophages/neutrophils initially mobilized by the invading pathogen. TNF-α and IL1h, in particular, are necessary to optimally activate differentiation of naïve T cells[69]. Thus, production of ROS ties the innate immune response to the adaptive immune response and is required for production of cytokines and other inflammatory molecules. Precise regulation of ROS production and activity is a key to down-regulation of the immune response; failure to do this can result in prolonged inflammation, which may be a factor in certain pathological conditions. Modulation of ROS levels by drugs or dietary intervention could have an impact on treatment of these conditions. As mentioned earlier, in a more general sense, additional work on the overall interaction of diet and drugs on the actual immune mechanism is absolutely necessary.

Role of ROS against cancer:

Production of free radicals in cancer cells seems a defense mechanism by which body tries to fight against cancer cells. Free radicals especially oxygen species provide a supply for hydrogen peroxide which eventually decomposes to water and oxygen. Oxygen, in turn, reduces neovascularization and metastasis[77]. Thus, cancer is a condition which is accompanied with a decrease in intracellular oxygen. It looks like that every drug or plant extract which can increase intracellular hydrogen peroxide and further decomposition to water and oxygen may be effective in treatment of cancer[79]. On the other hand, hydrogen peroxide can convert to hydroxyl anion and hydroxyl radical via a process called Fenton reaction. Thus, we need a compound to produce free radicals not scavenge free radicals to overwhelm cancer. Although beneficial role of antioxidants in cancer prevention has been well described, it is still in doubt whether antioxidants are effective in treatment of cancer or not. Theoretically, antioxidants scavenge superoxide anion radical and thus deplete the supply of intracellular hydrogen peroxide which may deteriorate cancer conditions. On the other hand, they scavenge hydroxyl radicals which have beneficial effects. Therefore, we believe that use of antioxidants during chemotherapy of cancer could diminish benefits of chemotherapy or radiation to the patient by scavenging free radicals. This is because radiation and most of anticancer drugs work by generating free radicals to destroy rapidly-dividing cancer cells[79].

**Role ROS in thyroid functions:**

Another beneficial role of ROS in health has been revealed by patients with a rare form of hypothyroidism. Hydrogen peroxide is a necessary cofactor for thyroperoxidase, the enzyme participating in a final step of hormone production (Fig. 11). The dual oxidase (DUOX2, encoded by DUOX2 gene; first identified in human thyroid gland); and probably also DUOX1, is the enzyme that generates the hydrogen peroxide required for thyroid peroxidase function; this theory is well supported by the
Role of reactive oxygen species in diseases:

In a normal healthy human body, the generation of pro-oxidants in the form of ROS and RNS are effectively kept in check by the various levels of antioxidant defense. However, when it gets exposed to adverse physicochemical, environmental or pathological agents such as atmospheric pollutants, cigarette smoking, ultraviolet rays, radiation, toxic chemicals, over nutrition and advanced glycation end products. AGEs in diabetes, this delicately maintained balance is shifted in favor of pro-oxidants resulting in oxidative stress. All the biological molecules present in our body are at risk of being attacked by free radicals. Such damaged molecules can impair cell functions and even lead to cell death eventually resulting in diseased states. It has been implicated in the etiology of several (>100) of human diseases \[^{[82]}\]few of them are summarized in Fig. 13.

Oxidative stress and cancer:

The development of cancer in humans is a complex process including cellular and molecular changes mediated by diverse endogenous and exogenous stimuli. It is well established that oxidative DNA damage is responsible for cancer development \[^{[83]-[85]}\]. Cancer initiation and promotion are associated with chromosomal defects and oncogene activation induced by free radicals. A common form of damage is the formation of hydroxylated bases of DNA, which are considered an important event in chemical carcinogenesis \[^{[83],[86]}\]. This adduct formation interferes with normal cell growth by causing genetic mutations and altering normal gene transcription. Oxidative DNA damage also produces a multiplicity of modifications in the DNA structure including base and sugar lesions, strand breaks, DNA-protein cross-links and base-free sites. For example, tobacco smoking and chronic inflammation resulting from noninfectious diseases like asbestos are sources of oxidative DNA damage that can contribute to the development of lung cancer and other tumors \[^{[83],[87]}\]. The highly significant correlation between consumption of fats and death rates from leukemia and breast, ovary, rectum cancers among elderly people may be a reflection of greater lipid peroxidation \[^{[88],[89]}\].

Oxidative stress cardio-vascular disease:

According to the World Health Report 2003, CVD accounted for 16.7 million of total global deaths and is believed to be the leading cause of deaths in developing countries \[^{[90]}\]. Cardio-vascular diseases are classified into many disorders such as coronary orischaemic heart disease IHD (heart attacks), cerebrovascular disease (stroke), hypertension (raised blood pressure), congenital heart diseases, rheumatic heart diseases, heart failure, peripheral vascular diseases and cardiomyopathies \[^{[119]}\]. CVD affect both young and old people and the risk increases with age and are similar for men and women \[^{[120]}\]. On a global scale, high blood pressure, tobacco use, high blood cholesterol (hypercholesterolemia), diabetes mellitus, physical inactivity, low fruit and vegetable intake, obesity, aging and alcohol consumption have been reported as leading risk factors of CVD \[^{[120]}\]. Nevertheless, these traditional known risk factors do not provide a full explanation for all cases of CVD\[^{[91],[93]}\]. Recent research has identified what could be seen as novel risk factors that may assist to identify persons or populations at risk of developing CVD \[^{[93]}\]. One such novel risk factor is free and hydroxyperoxide radicals which promote oxidative stress. It has been reported that...
cardiovascular diseases are typically characterized by elevated levels of ROS, endothelial dysfunction and proinflammatory states\textsuperscript{(94,99)}. As free and hydroxyperoxide radicals have the potential to damage biological compounds and structures such as proteins, membrane lipids, DNA and carbohydrates, they have thus been involved in the etiology and pathogenesis of CVD\textsuperscript{(93,96)}. The pathophysiology of CVD although multi-factorial seems to have a common underlying pathogenesis factor in oxidative stress which is mediated by free radicals and other reactive products of oxygen metabolism. In the vascular vessel, free radicals and other ROS participate in the oxidation of fatty materials (mainly LDL cholesterol) which are deposited into the inner vascular wall of the cardiovascular system\textsuperscript{(97-99)}. The resultant effect of such free radicals attack is mostly the induction of endothelial dysfunction which is an early feature of chronic inflammation, atherosclerosis and vascular diseases (hypertension)\textsuperscript{(94,99)}.

Oxidative stress and neurological disease:
Oxidative stress has been investigated in neurological diseases including Alzheimer’s disease, Parkinson’s disease, multiple sclerosis, amyotrophic lateral sclerosis (ALS), memory loss, and depression\textsuperscript{(100-103)}. In a disease such as Alzheimer’s, numerous experimental and clinical studies have demonstrated that oxidative damage plays a key role in the loss of neurons and the progression to dementia\textsuperscript{(103)}. The production of $\beta$-amyloid, a toxic peptide often found present in Alzheimer’s patients’ brain, is due to oxidative stress and plays an important role in the neurodegenerative processes\textsuperscript{(103)}.

Oxidative stress and pulmonary disease:
There is now substantial evidence that inflammatory lung diseases such as asthma and chronic obstructive pulmonary disease (COPD) are characterized by systemic. Oxidants may play a role in enhancing inflammation through the activation of different kinases and redox transcription factors such as NF-kappa $\beta$ and AP-1\textsuperscript{(104,105)}.

Oxidative stress and rheumatoid arthritis:
Rheumatoid arthritis is an autoimmune disease characterized by chronic inflammation of the joints and tissue around the joints with infiltration of macrophages and activated T cells\textsuperscript{(106,107)}. The pathogenesis of this disease is due to the generation of ROS at the site of inflammation. Oxidative damage and inflammation in various rheumatic diseases were proved by increased levels of isoprostanes and prostaglandins in serum and synovial fluid compared to controls\textsuperscript{(107)}.

Oxidative stress and nephropathy:
Oxidative stress plays a role in a variety of renal diseases such as glomerulonephritis and tubulointerstitial nephritis, chronic renal failure, proteinuria, uremia\textsuperscript{(107)}. The nephrotoxicity of certain drugs such as cyclosporine, tacrolimus (FK506), gentamycin, bleomycin, vinblastine, is mainly due to oxidative stress via lipid peroxidation. Heavy metals (Cd, Hg, Pb, As) and transition metals (Fe, Cu, Co, Cr)-induced different forms of nephropathy and carcinogenicity are strong free radical inducers in the body\textsuperscript{(108-111)}.

Oxidative stress and ocular disease:
Oxidative stress is implicated in age-related macular degeneration and cataracts by altering various cell types in the eye either photochemically or nonphotochemically. Under the action of free radicals, the crystalline proteins in the lens can cross-link and aggregate, leading to the formation of cataracts. In the retina, long-term exposure to radiation can inhibit mitosis in the retinal pigment epithelium and choroids, damage the photoreceptor outer segments, and have been associated with lipid peroxidation\textsuperscript{(113,114)}.

Oxidative stress and fetus:
Oxidative stress is involved in many mechanisms in the development of fetal growth restriction and pre-eclampsia in prenatal medicine. Some reports indicate that blood levels of lipid peroxidation products (F2-isoprostanes, MDA) are elevated in pre-eclamptic pregnancy and intra-uterine growth retardation and it has been suggested that ROS/RNS play a role in the etiology of these diseases. In pregnancies complicated by pre-eclampsia, increased expression of NADPH oxidase 1 and 5 isoforms which are the major enzymatic sources of superoxide in the placenta is seen\textsuperscript{(115-118)}.

Conclusion:
Recent decades have seen a surge of interest in the role of ROS in health and disease. From basic science research to clinical trials, the biomedical community has rapidly advanced toward a better understanding of ROS-metabolizing systems and their contribution to specific conditions. This review paper has discussed both the physiological and pathological roles of free radicals. An important perspective is that free radicals are not exclusively beneficial or exclusively detrimental. Rather, they need to be maintaining at appropriate levels to ensure physiological function while preventing pathological damage. Antioxidants, represent a potential treatment for pathological levels of free radicals but there are number of problems at present associated with the practical application of antioxidant therapy like choice of nature of antioxidants, dose and duration of therapy to be employed, delivery at specific site, specificity of targeting free radicals and direct assay of free radical/reactive oxygen species. Moreover, irrational and non judicial use of antioxidant can also increase the risk of potential toxicity, as many antioxidants can also act as prooxidants under a range of circumstances as well as it still difficult to ascertain that free radicals are cause or consequence of pathology and there are inadequate evidences to support therapeutic efficacy of various
antioxidant. Further research needs to be undertaken in order to understand the mechanisms of action behind free radicals.

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