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Cancer Promotors ROS

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ABSTRACT

Oxygen derived species such as superoxide radical, hydrogen peroxide, singlet oxygen and hydroxyl radical are well known to be cytotoxic and have been implicated in the etiology of a wide array of human diseases, including cancer. Various carcinogens may also partly exert their effect by generating reactive oxygen species (ROS) during their metabolism. Oxidative damage to cellular DNA can lead to mutations and may, therefore, play an important role in the initiation and progression of multistage carcinogenesis. The changes in DNA such as base modification, rearrangement of DNA sequence, miscoding of DNA lesion, gene duplication and the activation of oncogenes may be involved in the initiation of various cancers. Elevated levels of ROS and down regulation of ROS scavengers and antioxidant enzymes are associated with various human diseases including various cancers. ROS are also implicated in diabetes and neurodegenerative diseases. ROS influences central cellular processes such as proliferation a, apoptosis, senescence which are implicated in the development of cancer. Understanding the role of ROS as key mediators in signaling cascades may provide various opportunities for pharmacological intervention.The term cancer refers to more than hundred types of the disease. Almost every tissue in the body can spawn malignancies and some can yield several types. Cancer cells possess an even more insidious property to migrate from the site where they originate and form masses at distinct sites in the body. Cancer progression is a stepwise process where the initiated cells, nodules, polyp or the papilloma evolve further and become progressively more malignant. The genes implicated in malignancy are often modified forms of human genes. The activation of proto-oncogenes into oncogenes may contribute to malignancy. Mutations can also convert proto-oncogenes into carcinogenic oncogenes.

INTRODUCTION

A promoter is a chemical or substance that promotes the expansion of the initiated cell. A promoter is a chemical or substance that promotes the expansion of the initiated cell. Cancer is a disease characterized by uncontrollable cell growth. The causative factors include genetic factors, exposure to carcinogens, etc. Carcinogenesis is the development of cancer. The gene which cause cancer is ONCO GENE. Carcinogens activate the oncogene hence causing cancer. Cancer cells are abnormal cells and they have characteristics that can be associated with their ability to grow uncontrollably. Cancer cells are characterized by undifferentiated and uncontrolled cell division. Cancer cells are non-specialized, and divide uncontrollably. Cancer in situ is a tumor located in its place of origin. Malignant tumors establish new tumor distant from the primary tumors. . Cancer cells characteristics distinguish them from normal cells. They have abnormal nuclei with many chromosomal irregularities. They form tumors because they do not exhibit contact inhibition. They induce angiogenesis and cause nearby

blood vessels to form a capillary network that services the tumor. Types benign where the cancerous cells are surrounded by a fibrous membrane preventing metastasis. Cancerous: the cancerous cells are not surrounded by a membrane and so are free to move throughout the body, spreading the tumor to other organs/tissues. Cancer cells arise when the cells check systems e.g. Tumor suppressor proteins or checkpoints in the cell cycle, fail to recognize and repair DNA damage. This results in uncontrolled replication of the cell which results in a tumor. Answer. First, cells change to dysplasia, then hyperplasia, then cancer cells. Example of cancer promotors ROS (Reactive oxygen species).

Reactive Oxygen Species

Reactive oxygen molecules, also known as reactive oxygen species or, ROS, are metabolic products formed from two types of cells that are involved in production and metabolism – the endoplasmic reticulum and the mitochondria. Reactive oxygen molecules have numerous biological effects. They can destroy bacteria and destroy human cells. Their function is to serve as messengers between cells and in the process of homeostasis. Reactive oxygen molecules are produced continuously in all animals that breathe air. Because the normal metabolic path depends on the consumption and chemical use of oxygen, the production of reactive oxygen molecules is unavoidable.

Reactive oxygen molecules are different than normal oxygen molecules. They have been changed by the process of "oxidation" and are very unstable. Because they are unstable, they tend to react with anything that they come in contact with. When in contact with cells in the body or the DNA within those cells, the reaction can be damaging and causing cell death or DNA mutation. When exposed to environmental stress, such as heat or UV rays, the levels of ROS willincrease dramatically and damage cell structures. This damage is known as oxidative stress. ROS are also created from exogenous sources such as pollutants, tobacco, smoke, drugs, or ionizing radiation.

The positive effects of ROS on cell metabolism can be seen in the platelet responses to wound repair. Yet, an excessive amount of ROS has been indicated in the inflammatory reactions seen in patients with cardiovascular disease, the cochlear damage that leads to hearing impairment and congenital deafness, stroke, cancer, Alzheimer's disease, and heart attack. While it is important to limit the number of reactive oxygen molecules, they serve an important function in the cell, including the function of the thyroid and the cellular response to bacterial infection. Because of the danger is seen with reactive oxygen molecules, methods have been developed to counteract their effects.

Reactive Oxygen Species

Reactive oxygen species (ROS) are derived from the metabolism of molecular oxygen [3]. ROS include superoxide anion radical ($O_2^{-\cdot}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and the highly reactive hydroxyl radical (OH). The deleterious effects of oxygen are said to result from its metabolic reduction to these highly reactive and toxic species.

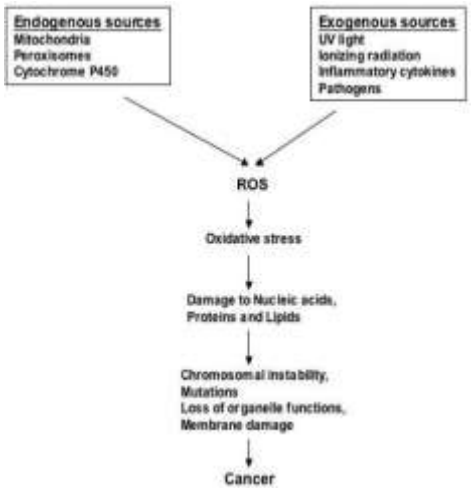
ROS normally exist in all aerobic cells in balance with biochemical antioxidants. Oxidative stress occurs when this critical balance is disrupted because of excess ROS, antioxidants depletion, or both. To counteract the oxidant effects and to restore redox balance, cells must reset important homeostatic parameters. ROS are not always harmful metabolic byproducts; when tightly regulated, ROS can act as intracellular signaling molecules.

In living cells, the major source of endogenous ROS are hydrogen peroxide and superoxide anion, which are generated as byproducts of cellular metabolism such as mitochondrial respiration. Alternatively, hydrogen peroxide may be converted into water by the enzymes catalase or glutathione peroxidase. Variability or inductive changes in the expression of these enzymes can significantly influence cellular redox potential. ROS can cause tissue damage by reacting with lipids in cellular membranes, nucleotides in DNA, sulfhydryl groups in proteins and cross-linking/fragmentation of ribonucleoproteins (see figure figure1).1). The relatively unreactive superoxide anion radical is converted by superoxide dismutase (SOD) into H_2O_2, which in turn take part in the "Fenton reaction", with transition metal ion (copper or iron) as catalysts, to produce the very reactive hydroxyl radical.

OXIDATIVE DNA DAMAGE AND CANCER

Damage to DNA by ROS has been widely accepted as a major cause of cancer. In patients with diseases associated with a risk of cancer indicates an increased rate of oxidative DNA damage or in some instances deficient repair system such as Fanconi anemia, chronic hepatitis, cystic fibrosis and various autoimmune diseases. Human studies support the experimentally based notion of oxidative DNA damage as an important mutagenic and apparently carcinogenic factor. ROS can damage DNA and the division of cells with unpaired or misreported damage leads to mutations. The majority of mutations induced by ROS appear to involve modification of guanine, causing G→T Trans versions.

If it relates to critical genes such as oncogenes or tumor suppressor genes, initiation/progression can result. Indeed, these species can act at several steps in multistage carcinogenesis. It is now assumed that ROS are involved both in the initiation and progression of cancer. Mutations caused by oxidative DNA damage include a range of specifically oxidized purines and pyrimidines, alkali labile sites, single strand breaks and instability formed directly or by repair processes. Because of the multiplicity of DNA modifications produced by ROS, it has been difficult to establish the frequency and specificity of mutations by individual oxygen radical induced lesions. Some of these modified bases have been found to possess mutagenic properties. Therefore, if not repaired they can lead to carcinogenesis. Studies show that although all the four bases are modified by ROS, mutations are usually related to modification of GC base pairs, while that of AT base pair rarely leads to mutations.



These mutations are usually base pair substitutions, whereas base deletions and insertions are less frequent. In human tumors, G to T Trans versions are the most frequent mutations in the p53 suppressor gene. Using single stranded DNA template in a sensitive forward mutation system, various mutations, including tandem double CC→TT substitution have been observed in DNA treated with oxygen free radicals.

Elevated levels of modified bases in cancerous tissue may be due to the production of large amount of H_2O_2, which has found to be characteristic of human tumor cells. Initiation of cancer in humans by ROS is further supported by the presence of oxidative DNA modifications in cancer tissue. Cigarette smoke, which is rich in carcinogens such as nitrosamines and polycyclic aromatic hydrocarbons, causes accumulation of 8-hydroxydeoxyguanosine (8-OHdG). Lungs from cigarette smokers contain two to three-fold higher 8-OHdG that could lead to mutations, some of which might be induced by oxygen free radicals, resulting in inflammatory responses, fibrosis and tumor development.

Urine obtained from smokers also has a four to tenfold elevation in altered nucleotides that are known to be produced by ROS. Urinary 8-OHdG is a biomarker of oxidative stress, cancer, atherosclerosis and diabetes. Oxidative DNA damage may be involved in the development of breast cancer. Increased steady-state levels of DNA base damage with a pattern characteristic of OH attack have been reported in inflammatory breast disease where malignant progression can occur. It is reported that elevated levels of 8-oxo-dG adducts in DNA play a fundamental role in breast cancer. Evidence also exists for the progression of breast tumor to the metastatic state and is an important etiologic factor.

Carcinoma of hepatic cells is often associated with chronic infection by hepatitis B or C viruses or ingestion of aflatoxin. Oxidative stress induced by these viruses represents one of the intracellular events that cause the genesis of hepatocellular carcinoma. G→T transition has been shown to be one of the more common types of mutation produced by aflatoxin lesion and ROS damage to DNA.

8-OHdG has also been reported to accumulate in hepatocellular carcinoma. The measurement of DNA damage and mutation in human liver as a function of persistence of chronic hepatitis mightbe predictive for the onset of liver cancer. Chronic prostate hypertrophy is diagnosed in most males by the age of 40 yr. But the late appearance of prostatic carcinoma suggests that a multistep process is involved in tumorigenesis. The paucity of known chemical agents associatedwith prostate cancer indicates an association with endogenous cellular process. The most reasonable candidates for endogenously formed endotoxins that accumulate in later life are the ROS.

The epidemiological studies involving measurement of typical modified DNA bases in a large variety of individual tumor tissue and their respective normal tissues may provide insights into the mechanism of carcinogenesis related to ROS. Measurement of purine and pyrimidine derived DNA lesions in tissues may prove to be useful in determining an association between free radicalproducing agents and cancer risk.

ROS AND DISEASES

There is growing awareness that oxidative stress plays a role in various clinical conditions such as malignant diseases, diabetes, atherosclerosis, chronic inflammation, viral infection,

and ischemia-reperfusion injury. ROS can cause oxidative DNA and protein damage, damage to tumor suppressor genes and enhanced expression of proto-oncogenes and oxidative stress has been shown to induce malignant transformation of cells in culture. Diseases associated with oxidative stress such as diabetes mellitus and cancer show a pro-oxidative shift in the redox state and impaired glucose clearance suggesting that muscle mitochondria are the major site of elevated ROS production. This condition may be referred to as 'mitochondrial oxidative stress'. Cancer patients commonly have decreased glucose clearance capacity, high glycolytic activity and lactate production. It is, therefore, suggested that the observed pro-oxidative shift is mediated by an increased availability of mitochondrial energy substrate. The 'inflammatory oxidative conditions' are typically associated with an excessive stimulation of NAD (P) H oxidase by cytokines and other factors. The increased ROS production or changes in intracellular glutathione levels are often involved with pathological changes indicative of a dysregulation of signal cascades or gene expression.

ROS are potential carcinogens because they facilitate mutagenesis, tumor promotion and progression. The growth promoting effects of ROS are related to redox-responsive cell signaling cascades. Sometimes, even normal cells show increased proliferation and expression of growth- related genes if exposed to H_2O_2 or $O_2^{-\cdot}$. Certain types of cancer cells also produce significant amounts of ROS. ROS production is induced after the expression of several genes associated with a transformed phenotype including H-Ras or mox1.

Because of its high metabolic rate and relatively reduced capacity for cellular regeneration, the brain is believed to be particularly susceptible to the damaging effects of ROS. In neurodegenerative diseases like Parkinson's, Alzheimer's and amyotrophic lateral sclerosis (ALS), ROS damage has been reported within the specific brain region that undergo selective neurodegeneration. Protein oxidation has been reported in the hippocampus and neocortex of patients with Alzheimer's disease, Lewy bodies in Parkinson's disease and within the motor neurons in ALS. Lipid peroxidation has also been identified in the cortex and hippocampus of patients with Alzheimer's disease, substantia nigra of patients with Parkinson's disease and spinalfluid in patients with ALS.

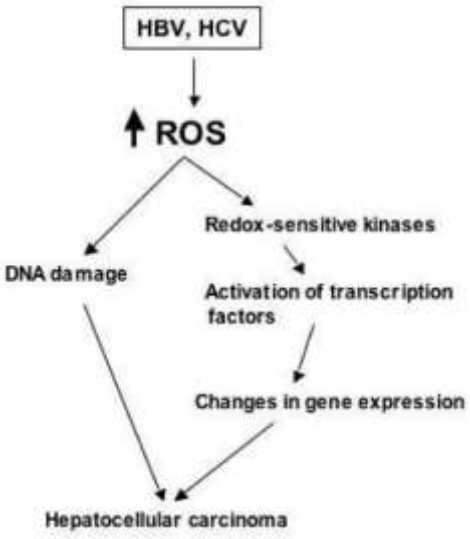
It is known that ROS can cause neuron and astrocyte death through apoptosis and necrosis. Mitochondria are involved in excitotoxicity nerve cell death through calcium-related bursts of ROS production and opening of permeability transition pores. Oxidative stress is also related to glutamate release and NMDA receptor activation during cerebral ischemia-reperfusion, production of $O_2^{-\cdot}$ In neurons and brain macrophages and glutamine-induced ROS production in astrocytes. Evidence implicating ROS in major degenerative diseases is also consistent with theirrole in brain aging. There is a general agreement that oxidative stress contributes to dopaminergic cell degeneration in Parkinson's disease. Oxidative stress has also been implicated as one of the earliest events in Alzheimer's disease.

ROS AND VIRAL INFECTION

Reactive oxygen metabolites play a complex role in many diseases and metabolic regulation. Because viruses replicate in living cells, such metabolites influence the growth of viruses in addition to serving as a host defense mechanism. Humans infected with viruses (HIV, hepatitis, and influenza) induce activation of phagocytes, which is associated with production of ROS. The activated phagocytes may also release pro-oxidant cytokines such as tumor necrosis factor (TNF)and interleukin-1.

Chronic hepatitis B (HBV) and hepatitis C virus (HCV) infections are associated with an increased production of ROS within the liver that is responsible for the oxidation of intracellular macromolecules. Infection with these viruses can also affect the host cell pro-/antioxidant balance by increasing cellular pro-oxidants such as iron and nitric oxide and also by inhibiting the synthesis of antioxidant enzymes. Antioxidants, together with agents interfering with the harmful effects of cytokines and lipid mediators, may have a role in the treatment of viral diseases.

ROS may facilitate or even promote replication of many viruses, depending on the cell and type of virus involved. Enhanced oxidative stress modulates the HCV RNA replication and hepatic cell survival via activation of oncogenic transcription factors that leads to the generation of hepatocellular carcinoma. Redox-sensitive kinases, Src, JAK, PI3K-Akt and MAPK (Erk, JNK, p38) regulate transcription factors through phosphorylation of the protein modules. Chronic HBV infection results in an increased total intra-hepatic iron and/or increase in the pro-oxidant low-molecular weight iron compartment of the liver. Previously, a strong correlation between the presence of HBV surface antigen and iron deposition in the Kuepfer cells and spleens of infected individuals has been reported. In addition to increased intracellular iron, elevated TNF-α has been found in hepatocytes from patients chronically infected with HBV.



Humans infected with HIV have been shown to be under chronic oxidative stress. HIV- seropositive humans exhibit decreased concentrations of naturally occurring antioxidant reductants such as total acid-soluble thiols, cysteine, and glutathione in plasma, peripheral blood monocytes, and lung epithelial-lining fluids. In addition, elevated levels of hydro peroxides and malondialdehyde are found in plasma of HIV-infected individuals. In cell culture system, ROS promotes replication of HIV, and antioxidants such as NAC inhibit the replication of the virus.

Oxidative stress has been reported to affect the cellular protein kinase/phosphatase balance, which is described in a number of tumors. The exogenous oxygen radical load is contributed by a variety of environmental agents (inhaled smoke and polluted air) and dietary antioxidants. Mutagens, tumor promoters and a variety of carcinogens including benzene, aflatoxin and benzo (a) pyrene may exert their partly by generating ROS during their metabolism.

ROS AND SIGNALING CASCADES

ROS is produced in non-phagocytic cells as a result of various signaling pathways such as receptor tyrosine kinases (RTKs) which become activated by growth factors – epidermal growth factor, platelet derived growth factor, fibroblast growth factor as well as cytokines (tumor necrosis factor, γ-interferon and interleukins) leading to an intracellular tyrosine phosphorylation cascade. The ROS activated signal transduction pathways are regulated by

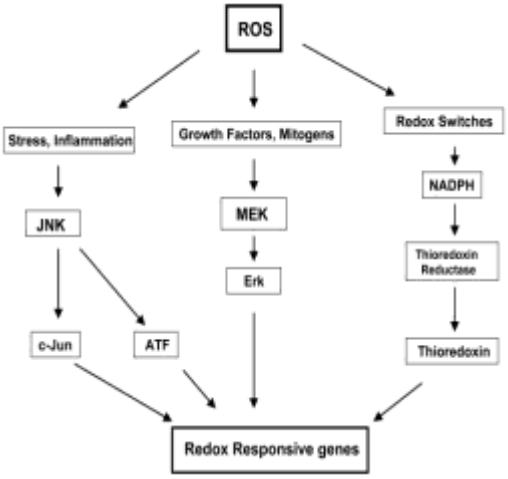
two distinct protein families – the Mitogen Activated Protein Kinase (MAPK) and the redox sensitive kinases. The MAPKs transduce signals from the cell membrane to the nucleus in response to a wide range of stimuli. MAPKs are serine/threonine kinases that, upon stimulation, phosphorylate their specific substrates at serine and/or threonine residues. Such phosphorylation events can either positively or negatively regulate substrate, and thus entire signaling cascade activity. Thus, the MAPK signaling pathways modulate gene expression, mitosis, proliferation, motility, metabolism, and programmed cell death. Conventional MAPKs consist of three family members: the extracellular signal-regulated kinase (ERK, subdivided into ERK1 and 2); the c-Jun NH2-terminal kinase (JNK, subdivided into JNK1, 2 and 3); and the p38 MAPK (subdivided into α, β, γ, and δ p38- MAPK.

MAPKs regulate processes important in carcinogenesis including proliferation, differentiation, and apoptosis. MAPK modulate gene expression through phosphorylation of a wide array of transcription factors. Of the three subfamilies, the ERK pathway has most commonly been associated with the regulation of cell proliferation. Activation of the ERK, JNK, and p38 subfamilies has been observed in response to changes in the cellular redox balance. The balance between ERK and JNK activation is a key determinant for cell survival as both a decrease inERK and an increase in JNK is required for the induction of apoptosis. Activation of MAPKs directly leads to increased AP-1 activity resulting in increased cell proliferation. One of thegenes regulated by AP-1 is cyclin D1. AP-1 binding sites have been identified in the cyclin D1 promoter and AP-1 activates this promoter, resulting in activation of cyclin-dependent kinase (cdks), which promotes entry into the cell division cycle. C-Jun also stimulates the progression into the cell cycle both by induction of cyclin D1 and suppression of $p21^{waf}$, a protein that inhibits cell cycle progression. Jun, considered a negative regulator of c-Jun-induced cell proliferation, represses c-Jun-induced cyclin D1 activation by the transcription of $p16^{INK4a}$, a protein that inhibits the G1 to S phase transition.

NF-κB activation has been linked to the carcinogenesis process because of its roles in inflammation, differentiation and cell growth. NF-κB regulates several genes involved in cell transformation, proliferation, and angiogenesis. Carcinogens and tumor promoters including UV radiation, phorbol esters, asbestos, alcohol, and benzo (a) pyrene are among the external stimuli that activate NF-κB. The expression of several genes regulated by NF-κB (bcl-2, bcl-x*L*, TRAF1, TRAF2, SOD, and A20) promotes cell survival at least in part through inhibition of apoptotic pathways. Expression of NF-κB has been shown to promote cell proliferation, whereas inhibition of NF-κB activation blocks cell proliferation. Additionally, tumor cells from blood neoplasms, and colon, breast, pancreas, and squamous cell carcinoma cell lines have all been reported to constitutively express activated NF-Kb.

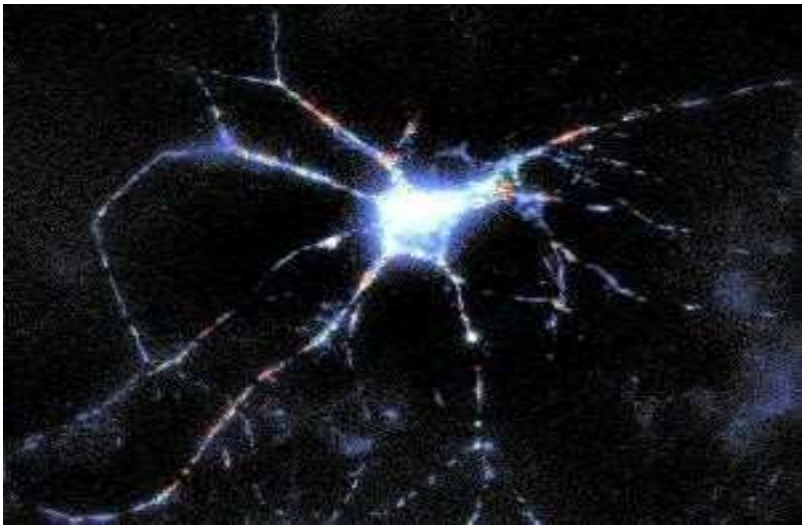
The second family consists of signaling factors that use cysteine motifs as redox-sensitive sulphydryl switches to modulate specific signal transduction cascades regulating downstream proteins. The redox-sensitive signaling cascade involves the cytoplasmic factors (thioredoxins), nuclear signaling factors such as Ref-1 (Redox factor-1) and transcription factors (AP-1, NF-κB, Nfr-1, and Egr-1). The cytoplasmic sulfhydryl containing proteins such as thioredoxins are critical upstream signaling proteins that regulate multiple intracellular processes such as DNA synthesis, cell growth, etc. The signaling cascades elicited by ROS culminates in the activation of c-Jun and c-Fos subunits of the active nuclear transcription factor, AP-1 (activator protein-1), that activate genes involved in

cellular proliferation. Redox-sensitive signaling factors regulate multiple processes including proliferation, cell cycle and anti-apoptotic signaling pathways. Inhibition of thioredoxins inhibits several pro-survival transcription factors such as Egr-1, AP-1 and NF-κBresulting in a G1 phase arrest.



–Reactive oxygen species, like many other things in our life, are beneficial and important in low levels, helping in cell regulation and function. But when those levels rise, they become detrimental and involved in disease processes from cancer to cardiovascular disease, ‖ says senior author GyorgyHajnoczky, MD, PhD, Professor of Pathology, Anatomy & Cell Biology and the leader of the MitoCare Center for Mitochondrial Imaging Research and Diagnostics at Thomas Jefferson University. –Neural damage and neurodegenerative diseases often produce prolonged elevations in reactive oxygen species. In these situations, halting mitochondria speeds up cell injury and can contribute to disease progression.

The various signaling cascades of ROS involved in the regulation and activation of redox sensitive genes. The role of reactive oxygen species in cell growth regulation is complex, being cell specific and dependent upon the form of the oxidant as well as the concentration of the particular reactive oxygen species. The modification of gene expression by reactive oxygen species has direct effects on cell proliferation and apoptosis through the activation of transcription factors including MAPK, AP-1, and NF-κB pathways. Oxidant-mediated AP-1 activation results in enhanced expression of cyclin D1 and cdks, which in turn promotes entry into mitosis and cell division. Likewise, reactive oxygen species function as second messengers involved in activation of NF-κB by tumor necrosis factor and cytokines. DNA damage, mutation, and altered gene expression are all required participants in the process of carcinogenesis. Although these events may be derived by different mechanisms, a common theme is the involvement of reactive oxygen species and oxidative stress in neoplastic transformation.



Reactive Oxygen Species Stop Mitochondria in Their Tracks

To satisfy the cell's energy needs, reactive oxygen species halt mitochondria by tampering with the molecular machinery that anchors these power-generating organelles to their motor proteins.

They illustrate that moderate and localized increases in reactive oxygen species can benefit neurons and other cells by temporarily detaining mitochondria in areas of need. Their research, published in *Cell Reports*, also sheds light on possible ways to treat immobilized mitochondria in neuronal injury and disease, when reactive oxygen species are exceedingly high.

Reactive oxygen species (ROS) are small, highly reactive, oxygen–containing molecules that are naturally generated in small amounts during the body's metabolic reactions and can react with and damage complex cellular molecules such as fats, proteins, or DNA. Alcohol promotes the generation of ROS and/or interferes with the body's normal defense mechanisms against these compounds through numerous processes, particularly in the liver. For example, alcohol breakdown in the liver results in the formation of molecules whose further metabolism in the cellleads to ROS production.

Alcohol also stimulates the activity of enzymes called cytochrome P450s, which contribute to ROS production. Further, alcohol can alter the levels of certain metals in the body, thereby facilitating ROS production. Finally, alcohol reduces the levels of agents that can eliminate ROS (i.e., antioxidants). The resulting state of the cell, known as oxidative stress, can lead to cell injury. ROS production and oxidative stress in liver cells play a central role in the development of alcoholic liver disease. Key words: alcoholic liver disorder; oxidative stress; free radicals; reactive oxygen species; chronic AODE (alcohol and other drug effects); NAD; NADH oxidoreductases; cytochrome P450; peroxidation; metals; proteins; DNA; lipids; glutathioneperoxidase; biochemical mechanism; survey of research. As described throughout the articles in this issue of *Alcohol Research & Health*, alcohol acts through numerous pathways to affect the liver and other organs and to lead to the development ofalcoholic liver disease (ALD) (for summaries of many of these pathways, see Cerebrum 2001; Bondy 1992; Nordmann et al. 1992). No single process or underlying mechanism can account for all the effects of alcohol on an organism or even on one specific organ; instead, many mechanisms act in concert, reflecting the spectrum of the organism's response to a myriad of direct and indirect actions of alcohol. One factor that has been suggested as playing a central role in many pathways of alcohol–induced damage, and which has been the focus of much research, is the excessive generation of molecules called free radicals, which can result in a state called oxidative stress. (These terms and concepts will be defined and explained in more detail in the following sections.) Particularly important are the actions of a class of oxygen–containing free radicals known as reactive oxygen species (ROS). ROS can damage or cause complete degradation (i.e., peroxidation) of essential complex molecules in the cells, including fat molecules (i.e., lipids), proteins, and DNA. Both acute and chronic alcohol exposure can increase production of ROS and enhance peroxidation of lipids, protein, and DNA, as has been demonstrated in a variety of systems, cells, and species, including humans.

Researchers have learned much about alcohol metabolism and the various enzymes and pathways involved, as well as about the role of lipid peroxidation and oxidative stress in alcohol toxicity. This article summarizes some of these findings. A detailed description of free

radicals, ROS, and oxidative stress is followed by a review of the alcohol–related cellular systemsinvolved in ROS production. Next, the article explains why ROS are toxic to cells and what systems have evolved to help cells protect themselves against ROS. Finally, the role of ROS and oxidative stress in alcohol–induced cell injury is discussed, with suggestions about future directions for research in this field. Although this discussion focuses on the role of oxidative stress in alcoholic liver disease, alcohol–induced oxidative stress also occurs in and damages other tissues (e.g., muscle, pancreas, and nerve cells).

What Are Free Radicals and ROS?

A free radical is an atom, molecule, or compound that is highly unstable because of its atomic or molecular structure (i.e., the distribution of electrons within the molecule). As a result, free radicals are very reactive as they attempt to pair up with other molecules, atoms, or even individual Electrons to create a stable compound. To achieve a more stable state, free radicals can ―steal‖ a hydrogen atom from another molecule, bind to another molecule, or interact in various ways with other free radical.

One chemical element frequently involved in free radical formation is oxygen. Molecular oxygen (O_2) is essential for cell function because it plays a pivotal role in a series of biochemical reactions occurring in the respiratory chain, which is responsible for most of the production of adenosine triphosphate (ATP), which provides the energy required for a multitude of cellular reactions and functions. (For more information on the respiratory chain and ATP production, see the article by Cunningham and Van Horn in this issue.). In the respiratory chain, which takes place in membrane–enclosed cell structures called mitochondria, an electron and a proton (H^+) are removed from a helper molecule (i.e., cofactor) called reduced nicotinamide adenine dinucleotide (NADH). (NADH is generated in the fluid filling the cell [i.e., the cytosol] and then moves to the mitochondria.) The electron is transferred to the first component of the respiratory chain, and the proton is released into the surrounding fluid. Chemically speaking, NADH is oxidized to NAD^+ in this reaction, whereas the respiratory chain, component that accepts the electron is reduced. (Oxidation reactions are those that add oxygen to a molecule or remove hydrogen or an electron from a molecule. The reverse reactions [i.e., removal of oxygen or addition of hydrogen or electrons] are called reductions.

The NAD^+ subsequently can be used again to accept new hydrogen atoms that are generated during the metabolism of sugars (e.g., glucose) and other nutrients. The reduced respiratory chain component, in turn, passes the electron on to other molecules in the respiratory chain until it is finally transferred to O_2, which then interacts with protons in cells to generate water. This series of electron transfer reactions generates sufficient energy to produce several molecules of ATP for each electron that passes through the respiratory chain.

Molecular oxygen can accept a total of four electrons, one at a time, and the corresponding number of protons to generate two molecules of water. During this process, different oxygen radicals are successively formed as intermediate products, including superoxide ($O_2^{\bullet-}$); peroxide ($O_2^{=}$), which normally exists in cells as hydrogen peroxide (H_2O_2); and the hydroxyl radical ($^{\bullet}OH$). Superoxide, peroxide, and the hydroxyl radical are considered the primary ROS and have sparked major research on the role of free radicals in biology and medicine. (Superoxide can react with itself to produce H_2O_2. Thus, systems producing superoxide also will result in formation of H_2O_2. Technically, H_2O_2 is not a free radical, but it is commonly

included among the ROS.) However, because they are unstable and rapidly react with additional electrons and protons, most of these ROS are converted to water before they can damage cells. It has been estimated that only about 2 to 3 percent of the O_2 consumed by the respiratory chain is converted to ROS. Nevertheless, the toxic effects of oxygen in biological systems—such as the breakdown (i.e., oxidation) of lipids, inactivation of enzymes, introduction of changes (i.e., mutations) in the DNA, and destruction of cell membranes and, ultimately, cells—are attributable to the reduction of O_2 to ROS.

What Is Oxidative Stress?

Because ROS form naturally during many metabolic processes, cells have developed several protective mechanisms to prevent ROS formation or to detoxify the ROS. These mechanisms employ molecules called antioxidants, which will be discussed in more detail in the section.

–Protection against ROS Toxicity. ‖ Under certain conditions, such as acute or chronic alcohol exposure, ROS production is enhanced and/or the level or activity of antioxidants is reduced. The resulting state—which is characterized by a disturbance in the balance between ROS production on one hand and ROS removal and repair of damaged complex molecules (such as proteins or DNA) on the other—is called oxidative stress. Oxidative stress is associated with numerous deleterious consequences for the cell (e.g., lipid peroxidation or even cell death), and alcohol– induced oxidative stress may play a significant role in the development of ALD. Many processes and factors are involved in causing alcohol–induced oxidative stress, including:

- Changes in the NAD^+/NADH ratio in the cell as a result of alcohol metabolism. Alcohol is metabolized in two steps. First, the enzyme alcohol dehydrogenase converts alcohol to acetaldehyde, a toxic and reactive molecule. Next, the enzyme aldehyde dehydrogenase converts the acetaldehyde to acetate. Each of these reactions leads to formation of one molecule of NADH, thereby providing more starting material and thus enhanced activity of the respiratory chain, including heightened O_2 use and ROS formation.
- Production of acetaldehyde during alcohol metabolism, which through its interactions with proteins and lipids also can lead to radical formation and cell damage. (For information on acetaldehyde and its detrimental effects, see the article in this issue by Tuma and Casey.)
- Damage to the mitochondria resulting in decreased ATP production.
- Effects on cell structure (e.g., the membranes) and function caused by alcohol's interactions with either membrane components (i.e., phosphate–containing lipids [phospholipids]) or enzymes and other protein components of the cells.
- Alcohol–induced oxygen deficiency (i.e., hypoxia) in tissues, especially in certain areas of the liver lobules (i.e., the paracentral region), where extra oxygen is required to metabolize the alcohol. (For more information on alcohol–induced hypoxia in the liver and its consequences, see the article by Cunningham and Van Horn in this issue.)
- Alcohol's effects on the immune system, which lead to altered production of certain signaling molecules called cytokines, which in turn lead to the activation of an array of biochemical processes. (For more information on alcohol's effect on cytokine production and its consequences, see the article in this issue by Neuman.)
- Alcohol–induced increase in the ability of the bacterial molecule endotoxin to enter the bloodstream and liver, where it can activate certain immune cells. (For more information on the role of endotoxin in liver damage, see the article by Wheeler in this issue.)

- Alcohol–induced increases in the activity of the enzyme cytochrome P450 2E1 (CYP2E1), which (as described in the section ‑Systems Producing ROS‖) metabolizes alcohol and other molecules and generates ROS in the process.
- Alcohol–induced increases in the levels of free iron in the cell (i.e., iron that is not bound to various proteins), which can promote ROS generation, as described in the section ‑Role of Metals.‖
- Effects on antioxidant enzymes and chemicals, particularly a molecule called glutathione (GSH),as described in the section ‑Protection against ROS Toxicity.‖
- Biochemical reactions generating an alcohol–derived radical (i.e., the 1–hydroxyethyl radical).
- Conversion of the enzyme xanthine dehydrogenase into a form called xanthine oxidase, which can generate ROS. Many of these processes operate concurrently, and it is likely that several, indeed many, systems contribute to the ability of alcohol to induce a state of oxidative stress.

Systems Producing ROS

As implied in the previous section, numerous cellular systems can produce ROS. The major source of ROS production in the cell is the mitochondrial respiratory chain, which, as described earlier, utilizes approximately 80 to 90 percent of the O_2 a person consumes. Thus, even though only a small percentage of that oxygen is converted to ROS, the mitochondrial respiratory chain in all cells generates most of the ROS produced in the body.

Another major source of ROS, especially in the liver, is a group of enzymes called the cytochrome P450 mixed–function oxidases. Many different variants of these iron–containing enzymes exist, some of which are responsible for removing or detoxifying a variety of compounds present in our environment and ingested (e.g., foods or drugs), including alcohol. Some cytochrome P450 enzymes also are important for metabolizing substances that naturally occur in the body, such as fatty acids, cholesterol, steroids, or bile acids. The biochemical reactions spurred (i.e., catalyzed) by the cytochrome P450 molecules use molecular oxygen, and during these reactions small amounts of ROS are generated. The extent of ROS generation may vary considerably depending on the compound to be degraded and on the cytochrome P450 molecule involved. One type of cytochrome molecule that is especially active in producing ROS is known as CYP2E1. This enzyme is of particular interest when investigating alcohol–induced oxidative stress because its activity increases after heavy alcohol exposure and because CYP2E1 itself also metabolizes alcohol.

ROS also are produced by a variety of oxidative enzymes present in cells, such as the previously mentioned xanthine oxidase. Under normal physiological conditions, xanthine oxidase acts as a dehydrogenase—that is, it removes hydrogen from xanthine or hypoxanthine and attaches it to NAD, thereby generating NADH. However, under certain conditions, such as the disruption of blood flow to a tissue, xanthine dehydrogenase is converted to a ROS–producing oxidase form. Alcohol consumption also may promote the conversion of xanthine dehydrogenase to xanthine oxidase, which can generate ROS, thereby enhancing oxidative stress.

Other sources of ROS in the body are two types of immune cells called macrophages and neutrophils, which help defend the body against invading microorganisms. In this case, however,ROS production is beneficial and even essential to the organism because it plays a

central role in destroying foreign pathogens (Rosen et al. 1995). Macrophages and neutrophils contain a group of enzymes called the NADPH oxidase complex, which, when activated, generates superoxide radicals and hydrogen peroxide. Hydrogen peroxide then interacts with chloride ions present in the cells to produce hypochlorite (the active ingredient in bleach), which in turn destroys the pathogen. The NADPH oxidase complex and the resulting ROS production are critical to the body's defense against all kinds of diseases, as is evident in patients with a condition called chronic granulomatous disease, in which ROS production by the NADPH oxidase complex is drastically reduced. Patients with this condition are highly sensitive to infections and usually die at an early age.

Besides the ROS generation that occurs naturally in the body, humans are constantly exposed to environmental free radicals, including ROS, in the form of radiation, UV light, smog, tobacco smoke, and certain compounds referred to as redox cycling agents, which include some pesticides, but also certain medications used for cancer treatment. The toxicity of these medications against tumor cells (as well as normal body cells) results from the fact that the compounds are modified by cellular enzymes to an unstable intermediate, which then reacts with molecular oxygen to produce the original product plus a superoxide radical. Thus, a vicious cycleof chemical reactions involving these compounds continually produces ROS.

Role of Metals

Most of the systems for the production of ROS described above produce superoxide radicals or hydrogen peroxide. Earlier studies suggested the possibility that these two radicals could interact with each other to produce the most reactive ROS, the hydroxyl radical ($^{\bullet}OH$). Under normal physiological conditions, direct interaction between these two radicals is not likely to play a significant role in generating hydroxyl radicals. However, in the presence of certain metals, particularly free iron or copper ions, a sequence of two reaction steps can occur that results in hydroxyl radical generation. In the first step, hydrogen peroxide can produce the hydroxylradical by removing an electron from the participating metal ion. (This reaction can generate other products as well, but the hydroxyl radical appears to be the primary oxidant generated. In the second step, involving the superoxide radical ($O_2^{\bullet-}$), the original metal ions are regeneratedso that they are again available for reaction with the hydrogen peroxide. This combination of two chemical reactions appears to account for most of the hydroxyl radical production in biological systems and explains, at least in part, why metals such as iron and copper produce oxidative stress and ROS–induced injury in cells.

Because of iron's critical contribution to hydroxyl radical formation, anything that increases the levels of free iron in the cells promotes ROS generation and oxidative stress. Chronic alcohol consumption has been shown to increase iron levels in the body not only when iron–rich alcoholic beverages, such as red wine, are consumed, but also because chronic alcohol consumption enhances iron absorption from food. Similarly, adding iron to alcohol–containing diets has been shown to exacerbate liver injury in animal studies, whereas administration of agents that capture free iron can prevent or ameliorate alcohol's toxic effects on the liver.

Why Are ROS Toxic?

ROS are toxic to cells because they can react with most cellular macromolecules, including proteins, lipids, and DNA. Proteins perform numerous crucial functions in the cell, primarily in the form of enzymes that mediate most biochemical reactions required for cellular

functions. Proteins are made up of approximately 20 different building blocks called amino acids, which differ in their sensitivity to interactions with ROS. For example, the amino acids cysteine, methionine, and histidine are especially sensitive to attack and oxidation by the hydroxyl radical. Accordingly, enzymes in which these amino acids are located at positions that are critical to the enzyme's activity will become inactivated by the interaction with ROS. Alternatively, the ROS– induced oxidation of proteins can lead to changes in the proteins' three–dimensional structure as well as to fragmentation, aggregation, or cross–linking of the proteins. Finally, protein oxidation often will make the marked protein more susceptible to degradation by cellular systems responsible for eliminating damaged proteins from the cell.

Lipids that contain phosphate groups (i.e., phospholipids) are essential components of the membranes that surround the cells as well as other cellular structures, such as the nucleus and mitochondria. Consequently, damage to the phospholipids will compromise the viability of the cells. The complete degradation (i.e., peroxidation) of lipids is a hallmark of oxidative damage. The polyunsaturated fatty acids[5] present in the membranes' phospholipids are particularly sensitive to attack by hydroxyl radicals and other oxidants. (Unsaturated fatty acids are those that contain a double bond between two of the carbon atoms making up the backbone of the fatty acid molecule. These double bonds can easily be opened in chemical reactions and interact with other substances. Fatty acids containing only one such double bond is called monounsaturated; fatty acids with two or more double bonds are called polyunsaturated.) A single hydroxyl radical can result in the peroxidation of many polyunsaturated fatty acid molecules because the reactions involved in this process are part of a cyclic chain reaction. In addition to damaging cells by destroying membranes, lipid peroxidation can result in the formation of reactive products that themselves can react with and damage proteins and DNA. (For more information regarding the actions of such reactive products.

DNA is the cell's genetic material, and any permanent damage to the DNA can result in changes (i.e., mutations) in the proteins encoded in the DNA, which may lead to malfunctions or complete inactivation of the affected proteins. Thus it is essential for the viability of individual cells or even the entire organism that the DNA remain intact. The building blocks of DNA molecules are called nucleotides; they consist of a sugar component and an organic base. Each DNA molecule consists of two strands of nucleotides held together by weak chemical bonds. Changes in the nucleotides in one strand can result in mismatches with the nucleotides in the other strand, yielding subsequent mutations. ROS are a major source of DNA damage, causing strand breaks, removal of nucleotides, and a variety of modifications of the organic bases of the nucleotides. Although cells have developed repair mechanisms to correct naturally occurring changes in the DNA, additional or excessive changes caused by ROS or other agents can lead to permanent changes or damage to the DNA, with potentially detrimental effects for the cell.

Protection against ROS Toxicity

Because ROS production is a naturally occurring process, a variety of enzymatic and no enzymatic mechanisms have evolved to protect cells against ROS. At least some of these mechanisms are impaired after long–term alcohol consumption and may therefore contribute to damage to the liver and other organs.

Protective Enzymes

Enzymes involved in the elimination of ROS include superoxide dismutase (SODs), catalase, and glutathione peroxidase. SODs catalyze the rapid removal of superoxide radicals. In mammals there are several types of SODs, which differ with respect to their location in the cells and the metal ions they require for their function. For example, a copper–zinc SOD is present in the fluid filling the cell (i.e., the cytosol) and in the space between the two membranes surrounding the mitochondria. Furthermore, a manganese–containing SOD is present in the mitochondrial interior (i.e., matrix). Both of these enzymes are critical for prevention of ROS–induced toxicity.(Another type of SOD [EC–SOD] is found outside the cells.) The effects of chronic alcohol exposure on the cellular content or activity of SODs are controversial, with reports of increases, no changes, or decreases, depending on the model, diet, amount, and time of alcohol feeding. Studies employing a commonly used model in which alcohol is administered directly into the stomach of laboratory animals (i.e., the intragastric infusion model, used most commonly with rats and mice) found decreases in SOD activity in the liver.

Catalase and the glutathione peroxidase system both help to remove hydrogen peroxide. Catalase is an iron–containing enzyme found primarily in the small membrane–enclosed cell components called peroxisomes; it serves to detoxify hydrogen peroxide and various other molecules. One way that catalase eliminates hydrogen peroxide is by catalyzing a reaction between two hydrogen peroxide molecules, resulting in the formation of water and O_2. In addition, catalase can promote the interaction of hydrogen peroxide with compounds that can serve as hydrogen donors so that the hydrogen peroxide can be converted to one molecule of water, and the reduced donor becomes oxidized (a process sometimes called the peroxidase activity of catalase). Compounds that can provide these hydrogen atoms include beverage alcohol (i.e., ethanol) and methanol.

The glutathione peroxidase system consists of several components, including the enzymes glutathione peroxidase and glutathione reductase and the cofactors glutathione (GSH) and reduced nicotinamide adenosine dinucleotide phosphate (NADPH).[7] ([7] Glutathione peroxidase contains an amino acid that is modified by addition of a molecule of the metal selenium; therefore, low amounts of selenium are critical for the body's antioxidant defense.) Together, these molecules effectively remove hydrogen peroxide. GSH, which consists of three amino acids, is an essential component of this system and serves as a cofactor for an enzyme called glutathione transferase, which helps remove certain drugs and chemicals as well as other reactive molecules from the cells. Moreover, GSH can interact directly with certain ROS (e.g., the hydroxyl radical) to detoxify them, as well as performing other critical activities in the cell.

No Enzymatic Mechanisms

Because of all its functions, GSH is probably the most important antioxidant present in cells. Therefore, enzymes that help generate GSH are critical to the body's ability to protect itself against oxidative stress. Alcohol has been shown to deplete GSH levels, particularly in the mitochondria, which normally are characterized by high levels of GSH needed to eliminate the ROS generated during activity of the respiratory chain.

Mitochondria cannot synthesize GSH but import it from the cytosol using a carrier protein embedded in the membrane surrounding the mitochondria. Alcohol appears to interfere with

the function of this carrier protein, thereby leading to the depletion of mitochondrial GSH.NADPH is involved in a much more diverse range of reactions in the cell than GSH. Nevertheless, because of its role in the glutathione peroxidase system, NADPH or the enzymes that generate this compound are sometimes considered antioxidants.

In addition to GSH and NADPH, numerous other no enzymatic antioxidants are present in the cells, most prominently vitamin E (α–tocopherol) and vitamin C (ascorbate). Vitamin E is a major antioxidant found in the lipid phase of membranes and, like other chemically related molecules, acts as a powerful terminator of lipid peroxidation. During the reaction between vitamin E and a lipid radical, the vitamin E radical is formed, from which vitamin E can be regenerated in a reaction involving GSH and ascorbate. Alcohol also appears to interfere with the body's normal vitamin E content because patients with ALD commonly exhibit reduced vitamin E levels.

Alcohol, Oxidative Stress, and Cell Injury

Excess levels of ROS and the resulting oxidative stress have been implicated in a variety of human diseases (see the sidebar). What is the evidence that alcohol–induced oxidative stress plays a role in cell injury, particularly damage to the liver cells? Many studies have demonstratedthat alcohol increases lipid peroxidation as well as the modification of proteins; however, it is not always clear if these changes are the causes rather than consequences of alcohol–induced tissue injury. Nevertheless, numerous investigations have found that administering antioxidants, agents that reduce the levels of free iron, or agents that replenish GSH levels can prevent or ameliorate the toxic actions of alcohol. For example, in the intragastric infusion model, the antioxidant vitamin E; the chemical ebselen, which mimics the actions of glutathione peroxidase; the copper–zinc or Manganese SODs; or a GSH precursor—all prevented ALD.

The most convincing data indicating that oxidative stress contributes to ALD come from studies using the intragastric infusion model. In these studies, ALD was associated with enhanced lipid peroxidation, protein modification, formation of the 1–hydroxyethyl radical and lipid radicals, and decreases in the hepatic antioxidant defense, particularly GSH levels (Knecht et al. 1995; Tsukamoto and Lu 2001; Iimuro et al. 2000; Nanji et al. 1994; Morimoto et al. 1994). Moreover, changes in the animals' diets that helped promote or reduce oxidative stress led to corresponding changes in the extent of liver injury. For example, when polyunsaturated fats (which are required for lipid peroxidation to occur) were replaced with saturated fats or other types of fats (i.e., medium–chain triglycerides), lipid peroxidation as well as ALD were reduced or prevented completely, indicating that both alcohol and polyunsaturated fats must be present for ALD to occur. The extent of the ALD was further exacerbated when iron—which, as mentioned earlier, is required for the generation of the hydroxyl radical and therefore promotes oxidative stress— was added to these diets (Tsukamoto et al. 1995). Conversely, the addition of antioxidants suchas vitamin E, SOD, or GSH precursors prevented the development of ALD, as mentioned above.

In addition to these studies conducted with intact animals (i.e., in vivo), studies with liver cells (i.e., hepatocytes) grown in culture also showed that alcohol can produce oxidative stress and hepatocyte toxicity. Studies with hepatocytes isolated from control rats or from rats that continuously had been fed alcohol indicated that alcohol metabolism via the enzyme alcohol dehydrogenase results in increased ROS production, hepatocyte injury, and a type of cell

death known as apoptosis. Moreover, all of these reactions could be blocked by the administration of antioxidants. Finally, studies using an established hepatocyte cell line that contains the alcohol– metabolizing and ROS–producing enzyme CYP2E1 demonstrated that adding alcohol, polyunsaturated fatty acids, or iron, as well as reducing GSH, resulted in cell toxicity, increased oxidative stress, and mitochondrial damage (Wu and Cerebrum 1999). Furthermore, all of these reactions could be prevented by administering antioxidants. Taken together, these findings indicate that alcohol–induced oxidative stress is a pivotal factor in the development of ALD.

Examples of ROS Role in Normal Physiological Processes

Role of ROS in Normal Vascular Diameter Regulation. Mitochondrial ROS, specifically superoxide anion ($O2\cdot-$) and hydrogen peroxide ($H2O2$), were demonstrated to play a role in normal vascular physiology in response to such factors as shear-stress. In the vascular system, ROS were demonstrated to originate mainly from the mitochondria in a study performed on human coronary resistance arteries. The mitochondrial origin of ROS was confirmed using electro biophysical methods that assesse the ROS generation and the response of vessel diameter to the presence of inhibitors of mitochondrial complexes and antioxidants. Go and colleagues have studied in more detail the mitochondrial role in the signaling response to oxidized milieu that might be encountered in the vascular system. Their results provide a model where by more oxidized environment in the plasma will lead to oxidation of cellular plasma membrane and cytoskeletal proteins. Oxidized proteins will then stimulate mitochondrial production of ROS that will initiate signaling pathways upregulating the cellular inflammatory response. The model described by Go et al. also provides explanation for the protective antioxidant role of small molecular weight-mitochondrial proteins such as thioredoxin 2 (Trx2). Earlier works have previously demonstrated Trx2 ‑regulatory‖ redox signaling pathway against mitochondrial ROS. The described model also provides partial explanation for the paradox that whereas moderate ROS levels contribute to regulation of vascular cell function, their excessive production is linked to pathological situations where redox damage and inflammation prevail in several chronic diseases. This kind of studies linking the cellular responses to alterations in redox potential on one hand to the intracellular signaling pathways on the other hand is promising; since it can be translated into designing novel therapeutic agents that target relevant signaling pathways as an alternative to the use of nonspecific antioxidant agents in clinical trials, or perhaps as a complementary tool to these agents.

Role of ROS in Oxygen Sensing

Oxygen sensing is so critical to cellular health as it allows cells to initiate adaptive responses that will increase the likelihood of survival in anticipation for limited oxygen availability. Guzy and Schumacker have proposed that the ETC acts as an $O2$ sensor by releasing ROS in response etohypoxia. The hypoxia induced released ROS act as signaling molecules that trigger diverse functional responses, among which is the increased production and stabilization of the hypoxia-inducible factor1 (HIF-1). This has been demonstrated at least in normal (no transformed) cells. As a matter of fact, a mutual regulation was reported for both HIF-1 and ROS. Under acute hypoxic conditions, the mitochondrial ETC produces excess ROS. This is required for the induction of HIF-1 expression, which in turn mediates adaptive metabolic responses culminating in a normalization of ROS levels and maintenance of redox homeostasis. Likewise, hypoxic induction of HIF-1 activity will end in normalization of the tissue $O2$ levels by stimulating angiogenesis is, which augments oxygen delivery to tissues

and solves the problem of tissue hypoxia. However, in some cancer cells the picture is not the same, as cells transformation is expected to result in alterations in the above-described normal adaptations, hence even though angiogenesis might occur, it is less effective in maintaining oxygen homeostasis; this wasextensively reviewed by Semenza.

Role of ROS in the Immune System

Essentially, ROS are deeply involved in both arms of the immunological defense system, the innate and the acquired responses. Upon exposure to environmental pathogens, exaggerated ROS production as a part of the oxidative burst in activated phagocytes present in the local inflammatory milieu represents one of the first lines of defense mounted against the invading pathogens. Although rapid, this innate immunity is usually only partially eff ective, since certain fraction of pathogens might escape and proliferate, thereby producing a larger number of pathogens. Acquired immunity will be initiated when pathogen-derived antigenic peptides that are the result of phagocytosis and digestion by activated phagocytes are presented to the T lymphocytes. As a result, the latter will proliferate and diff erentiate producing a large progeny ofimmunological eff ector cells that are capable of mounting an efficient and antigen-specificimmune response. ROS are involved in the acquired immune response because excess ROS continue to be locally produced by the activated phagocytes and consequently enhance the intracellular signal transduction cascades within the T lymphocytes and thereby decrease their activation threshold. The role exerted by ROS in immune responses will be revisited when the inflammation assembly as a part of chronic inflammation response will be discussed later in this review.

Role of ROS in Skeletal Muscle Physiology

The skeletal muscle is a target organ for oxidative regulation and/or oxidative stress since itrequires a large supply of energy to ensure efficient contraction, and consequently it is liable tobe exposed to excess mitochondrial ROS. The skeletal muscle production of ROS is promoted by multiple stimuli including muscle contraction, insulin, and hypoxia. Although under normal physiological conditions, antioxidant system control the level ofROS in skeletal muscle, oxidative stress can take place if ROS levels exceed the muscleantioxidant Journal of Biomedicine and Biotechnology.

Capabilities, and this can have damaging functional eff ects. Recent research has suggested that ROS can act as signaling intermediates in the regulation of skeletal muscle glucose uptake during contraction. However, results of such research have to be interpreted with caution as they have been somewhat in consistent depending on the model studied and the experimental design. Interestingly, muscle activity has been recently reported to affect the antioxidant defenses as well. Berzosa and colleague have reported an augmented effect of acute exercise in healthy untrained male subjects on the circulating total antioxidant status and antioxidant enzymes activities after both maximal and submaximal exercise periods. Others have shown that the elevated levels of antioxidant enzymes activity was also detected in various body organs. Thus, itis thought that exercise, whether acute or chronic, helps in maintaining redox homeostasis sinceit increases the antioxidant defense mechanisms, and due to the fact that long-term heavyexercise renders both animals and humans more resistant to oxidative damage. Not only muscle contraction has drawn the attention of scientists interested in the field of muscle physiology, but also muscle immobilization, where ROS production was reported to increase in skeletal muscle tissue after immobilization: a finding that warrant further studies specially if we consider that immobilized subjects manifest great loss of their

muscle mass.

3.2 Role of ROS in Genomic Stability

Regulation of Transcription, and Signal Transduction. Cellular redox status is considered an emerging regulatory factor for genomic stability and transcription. In a recent review article byRajendran and colleagues, the posttranslational enzymatic covalent modification of histone and no histone proteins in the form of acetylation/deacetylation for finely regulating transcription was discussed in relation to the cellular redox status. Various physiological processes such as cellcycle regulation, response to DNA damage, regulation of intermediary metabolism, programmed cell death, and autophagy, listing only few, are known to be regulated at the level of transcription of relevant genes. The author has reviewed in detail various factors regulating transcription via modulation of chromatin dynamics. They have indicated that oxidative stress and cellular energy consumption are among the key transcriptionregulating factors since the deacetylase activity of sirtuins, members of class III histone deacetylases, depends on the cellular redox status and NAD+ availability, respectively. In fact,the gene expression level of sirtuins has been shown to be under the control of the oxidative stress- and DNA damage-responsive transcription factor, E2F1, which regulates cell cycle and directly binds to the promoter of sirtuin 1, the most studied member of the sirtuins family. Moreover, exposure of cells to excess ROS such as H_2O_2 results in post translation modification of Sirt1 in the form of desumoylation and hence inactivation of Sirt1 deacetylation function, and consequently to acetylation and henceactivation of pro-apoptotic Sirt 1 substrates such as p53, and eventually cell death will take place. Under oxidative stress, the role played by ROS in transcription regulation is of critical importance and is able to affect vital processes such as glucose homeostasis, inflammation, cellular lifespan, and multiple aging-related diseases including cancer. Cells have an elaborate system to respond to redox status. This has been well studied in bacteria where the existence of a number of different ROS and redox status responsive signaling pathways is well established, as well as in the yeast Saccharomyces cerevisiae. In mammalian cells, similar yet incompletely understood protective redox responsive signal cascades have been described. These cascades are critical for the survival of cells which happen to be in the midst of highly oxidizing environment such as sites of infection and inflammation. While activated phagocytes utilize their capability of creating ―oxidative burst‖ to kill invading pathogens, and this implies the overproduction of ROS, recruited lymphocytes on the other hand need to possess an armament of oxidative stress-induced signal transduction cascades to protect themselves against this same oxidative burst. The oxidizing milieu modulates lymphocytes signal transduction cascades and increases the activities of redox-responsive transcription factors such as activator protein-1 (AP-1) and nuclear factor-κB (NFκB). The latter will bind and activate the promoters of various genes. One of those genesis the gene for the protective protein thioredoxin (Trx). Trx is an oxidoreductase that works together with the glutathione system for establishing and maintaining a reducing intracellular redox state. Other set of genes whose products are protective antioxidants are peroxiredoxin I (i.e., a Trx peroxidase), heme oxygenase-1, the cysteine transporter xc2, and manganese SOD (MnSOD).

―Potential Beneficial‖ Role of ROS in Cancer.

Recently an interesting hypothesis arises that examines the following question: ―Can antioxidants promote disease situations‖ or as Perera and Bardeesy stated it: ―When antioxidants are bad?‖ [4].This is a hot area of research and is finding increasing implications in cancer-related studies. Classically, ROS were demonstrated to promote

various types of cancers. This was explained by different facts: the ROS ability to induce DNA damage and thus to enhance therate of tumor-causing mutations and genetic instability, their pro-inflammatory effect, and their stabilizing influence on HIF essential for energy regulation. Accordingly, antioxidants were able to decrease tumori genesis by neutralizing the deleterious effects of ROS [20–23]. Recently, adifferent face of the ROS coin has been revealed based on studying the effect of mutations activating the transcription nuclear factor, nuclear factor-erythroid 2-related factor 2 (Nrf2). Nrf2is a redox stress-sensitive transcription factor that induces several antioxidant and detoxification genes. In the absence of redox stress states, Nrf2 is kept inactive by binding to another protein, Kelchlike ECH-associated protein 1 or KEAP1, ensuring effective Nrf2 repression. Somatic mutations in either Nrf2 or KEAP1 that prevent their binding will result in constitutive Nrf2 activation and transcription of Nrf2 target genes. Such mutations have been isolated from patients with lung cancer suggesting a protumorigenic role of Nrf2. Furthermore, drug resistance in some antitumor therapy may take place as a result of such somatic mutations; this was reviewed by Hayes. Journal of Biomedicine and Biotechnology and McMahon.

More recently, DeNicola and colleagues have demonstrated that in mice several endogenous oncogenes such as Kras, Braf, and Myc actively induce Nrf2 expression, promoting an ROS detoxification program and hence creating a more ‒reduced intracellular environment, a program that the authors suggest to be required for tumor initiation. As Hansson and Libby elegantly described the immune response in atherosclerosis as‒double edges word‖ the description seems to perfectly fit the ROS. Therefore, the big picture reflecting the contributions of various mediators plus local environmental factors seems to be the actual determinant for ROS-induced consequences in both physiology and pathology, and hence it is essential to unravel the not-yet-well understood parts of this intricate picture for better understanding of the ROS induced alterations.

Key Messages from Section 3. Although ROS have been classically known for their damaging effects, increasing evidence of their use in regulating and maintaining normal processes in living organisms has been accumulating. Therefore, the term redox regulation seems to better describe the redox status and its consequences. Both ROS and the protective antioxidant systems have to work in coordination to reach a state of redox homeostasis. Evidence of the roles played by ROS in several physiologic processes has been presented such as maintaining vascular diameter and normal vascular cell function, participating withHIF in sensing the oxygen availability and initiating responses appropriate for cell survival, mounting effective immune response, acting as possible signaling molecules in regulating skeletal muscle glucose uptake, and regulating gene stability and transcription via affecting chromatin stability. Antioxidants are equally essential, and their genes expression is regulated by the ROS. In addition, muscle exercise is beneficial in rendering us more resistant to oxidative damage. Recent evidence points out to a potential link between the‒reduced‖ cellular environment and tumor initiation.

4. ROS a Cellular Organelles Level

The Roles of the Endoplasmic Reticulum and Mitochondria in Oxidative Stress/Regulation. Both the endoplasmic reticulum (ER) and the mitochondrion have proven to be fascinating intracellular organelles that have stimulated a tremendous amount of research due to their unique characters. Their well-established roles in proper protein folding, posttranslational modifications,cellular trafficking, ions storage, energy production, cellular thermogenesis, and

intermediary metabolism are just some examples. Both organelles have strong and interrelated ties to the redox cellular homeostasis, disturbance of which is implicated in many diseases. Increasing evidence accumulates that ROS contribute to endothelial cell dysfunction, atherosclerosis, aging, and diabetes mellitus (DM) and diabetic complications, and CVD, to name only few.

4.1. Endoplasmic Reticulum and Endoplasmic Reticulum Stress. Impaired biological processes within the cell, collectively defined as cellular stress, together with chronic

Inflammation have been causally associated to various metabolic diseases, such as DM, obesity and CVD [25–31]. The ER, ubiquitously present in eukaryotic cells, plays a key role in protein folding and modification as well as in dynamic storage of calcium. It is through its role in maintaining protein folding that the ER is intricately involved in the overall ROS production as will be discussed shortly. Although protein folding is a multistep process that is not yet fully understood, two factors are known to be essentially required for the formation of intra- and intermolecular disulphide bonds that are fundamental to the folding process; these are the availability of energy and an ER oxidizing environment. In addition, two ER enzymes, the protein disulphide isomerase (PDI) and ER oxido reductin 1 (ERO1), are critical for the oxidative formation of disulphide bonds [32]. The reactions they catalyze involve transfer of electrons and oxidation of cysteine residues in nascent proteins and utilize flavin adenine dinucleotide (FAD) and molecular oxygen. Electron transfer to molecular oxygen as a terminal electron receiver produces H2O2; hence excess load of protein folding can result in accumulated ROS. The latter will trigger cellular inflammatory response. The ER is thought to sense signals of altered cellular states triggered by a variety of stimuli such as certain growth factors and hormones, limited availability of energy or nutrients, and the cellular redox state. The ER then acts accordingly aiming at restoring the normal cellular homeostasis. The ER itself might experience a state of ER stress, in which its capacity to correctly fold and modify proteins is overwhelmed by an excessive demand for protein folding or by conditions accompanied by excessive unfolded or misfolded proteins. This will increase the number of proteins of abnormal structure in the ER, triggering a defensive set of reactions collectively known as ‑unfolded protein response‖ or UPR, during which the cellular transcriptional and translational machineries are altered in order to restore the normal protein folding process. However, if the stress is extreme or prolonged, cellular homeostasis cannot be established and, alternatively, cellular pathways culminating. A less well-understood UPR system was recently described in the mitochondria (UPR mt) and its involvement in protecting cellular and specifically mitochondrial components against damaging consequences of metabolic stressors is increasingly acknowledged. At the molecular level, the relation between ER stress and oxidative stress can be explained by various routes. As mentioned earlier, during electron transfer to molecular oxygen as the terminal electron recipient in the ER protein folding process, some ROS will be generated.

Furthermore, under ER stress conditions, manifested by excess accumulation of unfolded or misfolded proteins, the cell consumes extra reduced glutathione (GSH) to correctly fold these aberrantly folded proteins, adding more to the cellular stress. Consequently, the ER stress can result in oxidative stress which as mentioned earlier might trigger an inflammatory state. Thus, it seems that the ER is placed in a vicious cycle where ER stress

can be caused by oxidative stress, and will also augment the perturbed oxidative redox state. Therefore, protective mechanisms essentially exist in the ER to limit the consequences of this damaging cycle. These include the protein kinase R-like ER Kinase (PERK) pathway-induced activation of an antioxidant program that utilizes the transcription factors: activating transcription factor-4 (ATF4) and Nrf2 [36–38]. As previously mentioned, activated Nrf2 will be translocated to the nucleus to increase the rateof expression of a group of antioxidant and oxidant detoxifying genes [39, 40].

4.2. Role of Mitochondria in ROS Production.

The mitochondrial ETC represents the major source for cellular ROS production; therefore, it is mentioned in various sections of this review. The superoxide anion is none enzymatically formed by the ETC semiubiquinone compound and then enzymatically converted into hydrogen peroxide by superoxide dismutase (SOD). Superoxide anion can also be non-enzymatically converted into hydrogen peroxide and singlet oxygen. Hydrogen peroxide can be converted into the highly reactive hydroxyl radical in the presence of reduced transition metals. Alternatively, hydrogen peroxide may be enzymatically converted into water by the enzyme's catalase or glutathione peroxidase [1]. Mitochondria possess several unique characters among which are the presence of mitochondrial DNA (mtDNA), their mode of inheritance, the dynamic nature of their structure, their indispensable roles in fuel metabolism and energy production, and the established links to various metabolic abnormalities. Therefore, it is expected that a defected mitochondrion is the underlying mechanism for a myriad of pathological conditions. The strong association between mitochondrial dysfunction, whether genetically determined or acquired, and chronic metabolic diseases such as type 2 DM (T2DM) and obesity was observed in many studies; yet a cause-effect relationship remained tentative for some time, till further studies demonstrated that impaired mitochondrial capacity and function are potential causes for insulin resistance and/or DM progression; this will be discussed below in more detail [41]. The central regulatory role played by the mitochondria in whole body metabolism, energetics, and homeostasis necessitates that it will be under tight control. Its ultimate functional capacity in certain tissue and under certain physiological conditions is the result of a network of interfering parameters. These include the mitochondrial DNA copy number, the mitochondrial density, and levels and activity of specific mitochondrial proteins [41]. Both transcriptional and posttranscriptional mechanisms exist to ensure tight control of the mitochondrial functional outcome. The nuclear DNA is deeply involved as well in implementing this control, and strong link between nuclear and mitochondrial gene expression was demonstrated more than 15 years ago [42]. As mentioned before, mitochondrial ETC is a potent source of ROS, and for obvious reasons such as the physical proximity to mtDNA, mitochondrial ROS generation is under tight control by various mechanisms, among which are the uncoupling proteins 1, 2, and 3 (UCP1, 2 and 3). UCPs are inner mitochondrial membrane proteins that are considered as natural regulators of mitochondrial ROS, responding.to and controlling ROS production by diminishing the formation of a large proton gradient [41].

It is thought that UCP1, which is present in the brown adipose tissue, evolved a thermogenic rolein mammals as a side pathway of the original, more general function of protecting cells against the cold-induced production of ROS. On the other hand, UCP2 (ubiquitously expressed at low levels) andUCP3 (preferentially expressed in skeletal

muscle) maintain their original function of decreasing ROS production through uncoupling and hence buffering ROS levels and do not appear to play a thermogenic role [43–45]. Other emerging roles of UCP have been suggested, for example, UCP2 is thought to exert a negative regulatory effect on pancreatic insulin secretion, as well as an ROS buffering effect on hypothalamic neurons controlling eating behavior; this will be detailed later [46,47]. Several years ago, the dynamic nature of the mitochondrial structure was elucidated and was demonstrated to be attained by complex molecular machinery, several components of which have been well characterized [48]. Abnormality in this machinery is linked to mitochondria—associated metabolic diseases. As an example, reduced expression of mitosis 2 (Mfn2), one of the mitochondrial proteins responsible for its dynamic morphology, was demonstrated to be partly responsible for decreased glucose oxidation and cell respiration in obesity.

Key Messages from Section 4. Both the ER and the mitochondria participate in maintaining normal cellular homeostasis. It is through the ER role in maintaining proper protein folding that this organelle is intricately involved in the overall ROS regulation. The ER senses signals of altered cellular redox states and then acts accordingly in order to restore and maintain normal homeostasis. During the UPR of the ER, ROS will be accumulated either due to actual production of ROS or due to consumption of the antioxidants such as GSH. Because the ER can be a part of a vicious cycle, where oxidative stress leads to ER stress, and the latter will further worsen the redox status, there are several protective mechanisms to limit the anticipated damage. A strong association and a potential cause-effect relationship exist between defective mitochondria and metabolic diseases. As in the ER case, several protective mechanisms exist to protect the mitochondria from oxidative damage. The antioxidants, as superoxide dismutase, catalase, and glutathione peroxidase/reductase system, are not it the scope of this review. UCPs are natural regulators for mitochondrial ROS, responding to and controlling the ROS production by diminishing the mitochondrial large proton gradient. Recently, UCP2 has been linked to other functions as well.

3. Role of ROS in Metabolic Diseases and Chronic Inflammation

Macromolecular ‒Toxicity ‖. In DM and obesity, the prevalent metabolic state is the one described by the term ‒glucolipotoxicity, ‖ in which excess extracellular glucose and Fatty acids (FAs) exert various damaging effects. Excess glucose increases oxidative stress through several biochemical mechanisms, including glyceraldehyde’s autoxidation, protein kinase C activation, glycation, methyl glyoxal and sorbitol production, hexamine pathway, and oxidative Phosphorylation [50]. Likewise, excess FA leads to peripheral insulin resistance and accumulation of lipid in no adipose tissue locations as the liver, heart, and pancreas, potentially resulting in failure of these organs. At the cellular organelles level, lipotoxicity has been recently linked to both oxidative and ER stress [51]. The linkbetween excess glucose and lipid—that is, macromolecules‖ cell stressors and inflammation was recently demonstrated in adipocyte where excess glucose and saturated FA, through ROS generation and activation of the nuclear transcription factor NF-κB, induced inflammation as manifested by upregulation of active inflammatory mediators involved in monocyte adhesion and chemotaxis. The Toll-like receptor 4 (TLR4) was implicated in mediating the effect of excess saturated FA—but not excess glucose— on the expression of these inflammatory mediators [52]. Moreover, and in contrast to excess saturated FAs, polyunsaturated FA were reported to exert anti-inflammatory effect on adipocytes that were linked to the nuclear receptor PPARγ [52]. These observations were supported by in vivo studies on experimental animals [53–55], but not yet in human.

5.2. Role of ROS in Insulin Resistance

Insulin resistance (IR) is not only a key feature of T2DM, but is also a characteristic of a wide range of clinical conditions such as obesity, metabolic syndrome, pregnancy, and sepsis [56]. IR can also occur, both in vivo and in vitro, as a consequence of certain experimental treatments with inflammatory cytokines such as tumor-necrosis factor-α (TNF-α) or with glucocorticoids such as dexamethasone, both treatments have obvious clinical implications. As a matter of fact, itis well established that elevated levels of TNF-α and/or glucocorticoids are detected in patients with the above-mentioned IR-associated clinical states [57–60]. Several factors have been demonstrated to play a role in IR. ROS hold a unique position among these factors, based on studies conducted on cell lines or in vivo. When the murine adipocyte cell line 3T3-L1 was treated with the ROS H2O2 or with ROS inducers, it clearly developed resistance to insulin [61, 62]. Moreover, markers of oxidative stress have been significantly associated with obesity, IR, DM, and sepsis [63, 64]. Similarly, conditions that increase ROS levels, for example, diseases with primary defects affecting ROS balance such as familial amyotrophic lateral sclerosis, were found to be associated with IR [65]. Albeit strong, the association between ROS and IR in various pathologic settings did not initially imply a cause-effect relationship; it just elucidated a strong association state. Nevertheless, such a causal effect was demonstrated few years ago by Houstis and colleagues. Using two approaches, cell lines (3T3-L1) and animal model of genetic obesity (ob/obmice), the authors have undoubtedly demonstrated that increased levels of ROS were indeed the cause for TNF-α- or dexamethasone-induced IR determined

By the lowered glucose uptake rate. Experimental intervening by either pharmacological agents or in transgenic animals designed to decrease ROS levels was shown to substantially prevent the IR status [56]. c-Jun NH2-terminal kinase (JNK) activation, which was detected upon stimulating the cell line with TNF-α or dexamethasone, was suggested to mediate the ROS-induced IR and was demonstrated to be linked to differential translocation of two important transcription factors; the pancreatic and duodenal homeobox-1 (PDX-1; which will be translocated from the nucleus to the cytosol thereby suppressing insulin biosynthesis) and the Forehead transcription factor Foxo1, which will be translocated in the opposite direction, from the cytosol to the nucleus, thereby contributing to insulin resistance by enhancing gluconeogenesis [66].Because the sphingolipid ceramide was reported to be increased in TNF-α- and dexameth as one induced IR in 3T3-L1 cell line and in diabetic muscle, it was suggested as a potential ROS source in insulin resistance[67– 69].

5.3 Role of ROS in Mitochondrial Dysfunction and in Diabetes Mellitus.

Normally, the β-cells of the pancreas adapt their insulin secretion to the fluctuations in blood glucose concentration sensed by their glucose sensor, glucosidase. During hyperglycemia, the rate of insulin-dependent glucose utilization by glycolysis in the β- cells will increase. Compared to other cell types, the β-cells manifest an unusually high proportion of glucose-derived carbon skeleton entering the mitochondria in the form of pyruvate that will they enter the tricarboxylic acid (TCA) cycle. Mitochondrial ETC promotes ATP generation, which will then be exported to the cytosol. Under high ATP:ADP ratio, the β-cells plasma membrane will be depolarized, and the potassium-ATP channels (KATP) will be closed allowing the opening of voltage-sensitive Ca2+ channels. Increased intracellular Ca2+ is the key trigger for exocytosis and insulin release from the secretory granules [70, 71]. This is referred to as stimulus-secretion coupling in the β-cells or glucose-stimulated insulin secretion (GSIS), as it

was initiated by glucose utilization. The pivotal role of normal mitochondrial ETC in the pancreatic β-cells glucose homeostasis has been established by a number of elegant studies over the past 30 years. Exposing the mitochondria to poisons or to restricted oxygen supply has established the finding that blockade of the mitochondrial ETC inhibits GSIS from β-cells [72].

This was later confirmed in experiments using rho0 β-cells, where the mtDNA-encoded subunits of the ETC enzymes are suppressed, while insulin biosynthesis and cell viability are preserved. The mitochondrial dysfunction in these cells and the consequent loss of mitochondrial ATP production have resulted in loss of GSIS. The lost response was restored by introducing normal mitochondria into the rho0 β-cells, confirming the mitochondrial origin of the defect [73]. The stimulus-secretion coupling in the β-cells was further studied in transgenic animals with β-cells targeted deletion of the nuclear encoded mitochondrial transcription factor (TFAM), which is the major transcription factor controlling the mtDNA genes expression. The βcells of this animal model manifest diabetic phenotype with both the ATP production and GSIS greatly diminished [76]. These animals represent a model for human mitochondrial diabetes, a rare form of DM that is maternally inherited, caused by mutations in the mtDNA, and usually associated with other pathological findings as bilateral sensory-neural deafness.

In patients with T2DM, the form of disease that affects almost 90% of all diabetic patients, somereports have demonstrated a decrease in the copy number of mtDNA in skeletal muscles and in peripheral blood cells [78, 79]. As previously mentioned, accumulation of ROS in the mitochondria (due to excessive production and/or defective defense mechanisms) is accompanied by mitochondrial dysfunction; this was found to be an age-related process [80]. Apparently, with advanced age the β-cells will be particularly susceptible to ROS damage, based on their low expression of the antioxidant protective enzymes, which will allow for the buildup of damaging effect of ROS [81, 82]. The mitochondrial uncoupling protein UCP2 is considered as a negative regulator of insulin secretion. Overexpression of UCP2 in β-cells diminishes ATP production and GSIS [46]. Likewise, deletion of UCP2 in mice enhances pancreatic islet ATP generation and GSIS. Furthermore, increased UCP2 in obesity was suggested to be one of the links between obesity and β-cell dysfunction in obesity induced T2DM [47]. However, the role ofUCPs is not fully understood, and specifically their response to the state of glucolipotoxicity that is highly manifested in uncontrolled DM and obesity requires further studies.

It is increasingly acknowledged that diabetic complications are also strongly linked to a state of oxidative stress. Diabetic retinopathy, being a major cause of blindness among adults worldwide,has been the focus of intensive research, which demonstrated that oxidative stress plays a vital role in its pathogenesis. In a recent review article, Zhu and Zou have presented data published from research studying the pigment-epithelium-derived factor (PEDF), which is a small secreted glycoprotein that was shown to exert protective effects on the retina based on its antioxidantproperties in addition to other functions as the neurotrophic, antiangiogenic, antivasopermeability, anti-inflammatory, and antifibrosis properties. Therefore, PEDF or its peptide derivatives might represent a potential therapeutic approach in the prevention and/or treatment of diabetic retinopathy, an area that still needs further assessment.

5.4 Role of ROS in Obesity and Obesity-Associated Comorbidities

Obesity—defined as a body mass index of 30Kg/m2 or higher—is a chronic disease with serious adverse consequences and is currently a leading cause of preventable deaths worldwide. It is an established independent risk factor for CVD [84]. Obesity is also associated with a state of chronic inflammation in the adipose tissues as well in other organs, where tissue- infiltrating monocytes/macrophages increase in number and inactivity. Several active mediators, chemotactic molecules, cytokines, and adipokines, augment the chronic inflammatory state and result in the excessive production of ROS causing systemic oxidative stress.

This is considered a Potential mechanism linking obesity, vascular abnormalities, and the elevatedrisk of atherosclerosis and CVD. One of the main sources of ROS in those situations is believed to be the NADPH oxidase (Nox), a multiprotein complex that is expressed both in phagocytes and endothelial cells. Feeding mice high-fat diet for 22 weeks to cause diet induced obesity was associated with activation of Nox. The latter is believed to elevate the expression of TLR in the vascular tissues, and probably in adipocytes as well. TLR4, which is the receptor for endotoxin and lipid, and its intracellular signaling consequences induce overexpression of proinflammatory cytokines, as TNF-α and IL6, and of transcription factors such as NF-κB. Therefore, Nox-induced elevated TLR4 expression and signaling might be involved in the obesity-induced inflammation and insulin resistance. Such findings propose the components of Nox system as potential novel therapeutic targets for obesity-associated comorbidities.

In recent years, novel roles have been assigned to the ROS as they relate to the central nervous system control over our body weight. The site of these new roles for ROS is the hypothalamus, where there are neurons controlling our satiety and others controlling our hunger behavior. Such roles have been implicated as contributing factors underlying diverse findings such as the age-related decrease ability to lose weight and the caloric restriction-induced longevity. Interesting findings demonstrated that different hypothalamic neurons have distinct preference to fuel utilization, so that glucose is the preferred fuel for proopiomelanocortin (POMC) neurons that are responsible for satiety, while FAs are preferred fuel to neuropeptide Y/Agouti-related in protein (NPY/AgRP) neurons responsible for feeding. Although ROS are produced in both types of neurons as a result of oxidation of glucose and FA, yet it wasdemonstrated that the ROS produced in the POMC neurons will be accumulating and henceimpairing the POMC neurons over time and this is thought to be responsible, at least in part, forour inability to lose weight as we get older. On the other hand, the ROS produced in theNPY/AgRP neurons that are active during negative energy balance will be buff ered by UCP2and this is thought to play a role in the mechanism of longevity induced by caloric restriction. This delicate neuronal system, although not completely well-understood, emphasizes the real need to be extra cautious with the use of any ant obesity pharmacological approach attempting topromote satiety or suppress hunger at the hypothalamic level.

5.5.2. Role of ROS in Infection.

ROS production has been used by human cells to fight infection, both bacterial and viral. Although the bactericidal eff ect of ROS is known since the 50s of the last centuries [94], active research in this area is still ongoing, especially with the aim to discover novel agents targeting bacterial strains with multiple antibiotic resistance, a serious clinical problem that is increasingly encountered. Recent experimental methodologies have been applied to this area;

for instance, a genome-wide transcriptional profiling of the response of Staphylococcus aureus (S. aureus) to cryptotanshinone, a medicinal plant-isolated chemical agent exhibiting antimicrobial activity against a broad range of bacteria [95]. Cryptotanshinone (CT) demonstrated effective in vitro antibacterial activity against all S. aureus strains tested. Asymetrix Gene Chips were used to determine the global transcriptional response of S. aureus to treatment with sub inhibitory concentrations of CT. Both antibacterial and active oxygen radical generation functions of CT were positively correlated. Moreover, the S. aureus was found to undergo a defensive oxygen- limiting state upon exposure to the drug. Hence, the authors suggested that both actions of the drug, the antibacterial and the oxygen radical generation, may be responsible for its pharmacologic efficiency.

This Type of studies is promising since it sets the platform for developing and characterizing novel antibacterial agents with optimum activity against antibiotic resistant bacterial strains. The ROS involvementin viral infection has also been studied since quite a long time (late 1980s and early 1990s) [96]; moreresearch is still being conducted and producing interesting results, especially in the field of human immune deficiency virus-1 (HIV-1) infection and treatment. HIV-1 infection is known to be associated with a state of oxidative stress. Interestingly, HIV-1 treatment using highly active antiretroviral therapy (HAART) seems to worsen the oxidative stress status. This was recently published by Mandas and colleagues who have compared the HIV-1-infected patients treated with HAART with untreated patients and with normal control. Moreover, optimal adherence to the HIV-1 therapy further worsened the oxidative stress status as compared to poor adherence [97]. More recently, higher oxidative stress status was demonstrated in patients coinfected with HIV-1 and HCV as manifested by higher oxidized glutathione level and more severe mitochondrial DNA damage as compared to patients who are monoinfected with HIV-1 [98].

The biochemical consequences that will take place include suppression of insulin biosynthesis and activation of gluconeogenesis, both will enhance the progression of IR into diabetes. In normal β-cells, there is a glucose-stimulated insulin secretion that is dependent on the level of ATP production and is diminished by the mitochondrial UCP2. The age-dependent mitochondrial dysfunction is particularly important in the β-cells due to their relative deficiency of antioxidant protective enzymes. The redox state will not only affect the incidence of DM, but it is also involved in the incidence of diabetic complications. PEDF is believed to be protective against the occurrence of diabetic retinopathy and hence is suggested to be of therapeutic potential and must be further investigated. Activation of Nox enzyme is believed to elevate the expression of TLR4 in vascular tissues and is involved in the obesity-induced inflammation and associated vascular abnormalities. ROS exert different effects on the hypothalamic neurons involved in satiety or hunger behaviors; therefore, caution should be exerted with attempting to design anti-obesity approach working at the hypothalamic level. Journal of Biomedicine and Biotechnology. Both bacterial and viral infections have been related to ROS generation. Novel approaches are utilized to develop antibacterial agents with optimum activity.

6. Antioxidant Therapeutics

Several natural antioxidants have been investigated in vitro or in animal models to assess their potential therapeutic effects in conditions linked to oxidative stress. Interestingly not all antioxidants are identical, results from recent studies emphasize that point, and some will be briefly summarized in the following section. In order to determine the protective role of

vitamin E and/or dithiothreitol (DTT), Tsai and colleagues have studied rat hepatocytes that have been exposed to oxidative stress by treating them with Tert-butyl hydro peroxide and have assessed the cellular calcium homeostasis in these cells. Their results indicated that vitamin E not only blocks the elevation of intracellular ionic calcium ions but also prevents the loss of protein thiols from the cellular membranes, leading the authors to suggest that vitamin E conserves the integrity of cell membranes and this might be important for the maintenance of intracellular calcium homeostasis [99]. Another natural antioxidant, rottlerin, was studied by Maioli et al., in human breast cancer and human colon cancer cell lines, MCF-7 and HT-29, respectively.

Rottlerin is a pigment that exerts a pleiotropic inhibitory effect on specific intracellular kinases and hence is thought to interfere with the NF-κB activation process. Similar polyphenolic phytochemical compounds as curcumin, resveratrol, and mangiferin were also reported to exert antioxidant activity that is mediated by NF-κB inhibition [101]. Because not all the antioxidant phytopolyphenols are identical in their mechanism of action, resveratrol and rottlerin, both are antioxidants that act as protein kinase C δ(PKC δ) inhibitors, inhibit NF-κB via different mechanisms. In addition, rottlerin exerts a free radical scavenging eff ect [100]. Similar to rottlerin, curcumin, which is commonly used as food additive in many parts of the world, exerts anti-inflammatory and antioxidant eff ect by scavenging free radicals and inhibiting NF-κB. Curcumin also inhibits lipid peroxidation as manifested by decreasing the hepatic malondialdehyde (MDA) level in a rat model of alcoholic liver disease. Samuhasaneeto and colleagues have induced liver injury in rats by feeding the methanol and the assessed the protective effect of orally administered curcumin. On the other hand, and at least in this studied model, curcumin did not affect the SOD activity nor did it affect the PPARγ protein expression level. Curcumin seems to inhibit the early stages of alcohol liver disease in rats. As a matter of fact, early stages of the disease are mainly linked to oxidative stress that is induced by excessive accumulation of ROS. To a lesser extent, curcumin was found to decrease hepatocytes apoptosis that is caused by mitochondrial dysfunction and cytochrome C release [101]. While curcumin did not affect the level of SOD activity, another natural antioxidant and anti-inflammatory compound, the purple sweet potato color (PSPC), was recently reported to increase the activity of Cu2+/Zn2+ SOD, as well as of catalase. This was reported in the brain tissue of a DGal-induced mouse model for aging, where Shan et al. first evaluated the animal spontaneous behavior and its cognitive performance and then thoroughly evaluated the biochemical changes taking place in the animal brain.

In this model, oral administration of PSPC resulted in improvement in the mice behavior and cognitive performance in the intact animal. At the level of the animal isolated brain tissues and in addition to the increased activity of Cu2+/Zn2+ SOD and catalase; the demonstrated low expression levels of induced NOS (iNOS) and of cyclooxygenase 2 (Cox2), the decreased nuclear translocation of NF-κB, and the lowered content of MDA have all led the authors to suggest that PSPC, through its antioxidant and anti-inflammatory capacity, ameliorates the cognition deficits and attenuates oxidative damage and inflammation in aging mouse brain. Ginsenoside Rb1, a natural plant steroid belonging to the family of glycosides and triterpene saponins, was recently reported by Xia and colleagues to attenuate the myocardial oxidative stress and tissue histological damage in a model of streptozotocin-induced diabetes and myocardial ischemia/reperfusion injury. Since this protective effect was abolished by the eNOS inhibitor, L-NAME, it was suggested that ginsenoside Rb1 exerts its protective effect by enhancing the expression of eNOS and hence increasing the NO content,

in addition to its antioxidant effect.

Interestingly, not all anti-inflammatory agents are antioxidants as well; diclofenac, a nonsteroidal anti-inflammatory drug (NSAID) that is usually prescribed to treat pain, fever, and inflammation is a clear example. It was recently reported that diclofenac resulted in apoptosis of neuroblastoma cell line. Diclofenac-induced apoptosis was related to its ability to cause mitochondrial dysfunction in the form of lowering the mitochondrial membrane potential and consequently releasing cytochrome, and eventually causing cellular apoptosis. The diclofenac- induced mitochondrial dysfunction was related to its prooxidant activity since it was found to decrease the protein level and activity of mitochondrial SOD, though not its mRNA level. Furthermore, exogenous administration of the antioxidant Trx lowered the diclofenac-induced apoptosis and improved the mitochondrial SOD protein level.

Such research has the potential to be of clinical significance as it can be applied in determining the optimum dosage and avoiding side effects and drug interactions caused by diclofenac [104]. Epoetin δ is an erythropoietin that is prescribed to patients who are at increased risk of developing anemia. It is unique because, unlike other erythropoiesis-stimulating agents, epoetinδ is produced by gene-activation technology in a human cell line, and hence it has a human-type glycosylation profile. The antioxidant capacity of epoetin δ was recently assessed in primary human renal tubular cells.

Journal of Biomedicine and Biotechnology Oxidative stress was first induced by treating the cellswith glucose oxidase enzyme. The protective antioxidant capability of epoetin δ was then assessed using a commercial oxidative status indicator (2, 7–dichlorodihydrofluorescein diacetate; H2DCFDA). The authors have demonstrated that epoetin δ antioxidant capacity has protected the renal tissue through upregulation of several renoprotective genes, some of them, as carboxypeptidase M, dipeptide peptidase IV, and cytoglobin, were reported for the first time tobe involved in the antioxidant renoprotection process.

6. Potential Novel ROS Targeted Therapeutics

Taken together, the results of recently conducted research studying the molecular, subcellular organelles, and cellular mechanisms involved in mediating the ROS actions off er promising venue as they propose novel potential therapeutic agents for the ROS-linked diseases. Few examples were presented in this review that should be further studied. The complexity and multifaceted nature of the process of redox regulation make it essential to better understand the key players in the process and then to design a targeted means of controlling these players. An obvious example is the JNK signaling pathway, which is activated by various cell stressors including ROS, glucolipotoxicity, and ER stress [106–108]. In the case of chronic ER stress, such as that seen in obesity, the ER stress-induced metabolic disturbance would result in insulin resistance and, ultimately, T2DM. Could inhibitors for JNK signaling pathway be designed to specifically ameliorate the ER-stress associated activation of this pathway? Results published by ¨Ozcan and colleagues have suggested that interventions that regulate the ER stress response off er new opportunities for preventing and treating T2DM. In addition, the serine/threonine kinase, Kappa kinase β (IKK) pathway is also activated by such stressors and is strongly involved in the development of β cell dysfunction, insulin resistance, and T2DM.

Therefore, it is possible that such pathway could be targeted as an approach that is

complementary to the classical antioxidants in the prevention and/or treatment of ROS-associated chronic diseases. However, this approach is usually neither predictable nor straightforward; therefore in vitro, as well as experimental animal models studies have to be conducted first, and based on their results, carefully designed human intervention studies could be proposed. Even with such design, the hypothesis of targeting a specific signaling pathway with the objective of ameliorating the redox stress-associated diseases remains subject to either approval or refutation. The recent published work by Meijer and colleagues is a clear case for the inherent complexity of metabolic disorders. Based on results from animal studies implicating that the transcription factor activator protein-1 (AP- 1) proinflammatory pathway is a promising target in the treatment of vascular diseases as atherosclerosis, this group has evaluated the profile of AP1 activation in human aortic wall samples and tested the potential benefit of AP-1 inhibition in a clinical trial involving patients with symptomatic peripheral arterial disease.

Using doxycycline (an AP-1 inhibitor) or placebo in those patients did not affect any of the markers of inflammation and vascular dysfunction, except for the C-reactive protein which only revealed a borderline reduction in the group treated with doxycycline. This review summarizes the key roles played by ROS, which are considered major redox species, although not the only ones; the thiol/disulfide redox system plays key roles as well in redox signaling and oxidative stress.

In fact, the limited benefit of the classical antioxidant therapeutic agents used so far in several clinical trials might be the result of the untargeted approach of these agents as mentioned above and importantly due to the fact that they are not affecting the cysteine-based redox regulators. Further research is indeed required for better clarifying the big picture of redox regulation both by ROS and non-ROS mediators. Ongoing research is being conducted to better understand the mechanism of action of known antioxidant agents and to design and test novel therapeuticagents. Has to be undertaken utilizing in vitro and in vivo animal models and human trials; nevertheless, results might not be predictable.

CONCLUSION

In conclusion, oxidative regulation is a term that better describes the actions of ROS, as some of these actions are considered physiological and others, especially if uncontrolled, are deeply involved in so many pathological situations. There is growing evidence that redox regulators, related active mediators, cellular organelles functions, and surrounding environments are all tied together in intricate networks affecting the whole body energetic, metabolism, state of health and disease and even lifespan. Although at present the use of antioxidants seems disappointing in preventing the progression of the ROS associated diseases, current research findings have proposed novel targets that might prove to be more appropriate antioxidants. Further research is needed to investigate the possible preventive and/or therapeutic values of these molecules.

Abbreviation ROS: Reactive oxygen species ETC: Electron transport chain Nox: NADPH oxidases H2O2: Hydrogen peroxide Trx2: Thioredoxin 2 HIF-1: Hypoxia-inducible factor-1 Nrf2: Nuclear factor-erythroid 2-related factor 2 CVD: Cardiovascular diseases DM: Diabetes mellitus

Top 10 most unhealthy, cancer-causing foods - never eat these again!

(Natural News) The statement "everything causes cancer" has become a popular hyperbole, and one that some people use as rhetorical fodder to excuse their own dietary and lifestyle failures, particularly as they pertain to cancer risk. But the truth of the matter is that many common food items have, indeed, been scientifically shown to increase cancer risk, and some of them substantially. Here are 10 of the most unhealthy, cancer-causing foods that you should never eat again.

1) **Genetically-modified organisms (GMOs)**. It goes without saying that GMOs have no legitimate place in any cancer-free diet, especially now that both GMOs and the chemicals used to grow them have been shown to cause rapid tumor growth. But GMOs are everywhere, including in most food derivatives made from conventional corn, soybeans, and canola. However, you can avoid them by sticking with certified organic, certified non-GMO verified, and locally-grown foods that are produced naturally without biotechnology (http://www.naturalnews.com).

2) **Processed meats**. Most processed meat products, including lunch meats, bacon, sausage, and hot dogs, contain chemical preservatives that make them appear fresh and appealing, but that canalso cause cancer. Both sodium nitrite and sodium nitrate have been linked to significantly increasing the risk of colon and other forms of cancer, so be sure to choose only uncured meat products made without nitrates, and preferably from grass-fed sources (http://www.organicconsumers.org/foodsafety/processedmeat050305.cfm).

3) **Microwave popcorn**. They might be convenient, but those bags of microwave popcorn are lined with chemicals that are linked to causing not only infertility but also liver, testicular, and pancreatic cancers. The U.S. *Environmental Protection Agency* (EPA) recognizes the perfluorooctanoic acid (PFOA) in microwave popcorn bag linings as "likely" carcinogenic, and several independent studies have linked the chemical to causing tumors. Similarly, the diacetyl chemical used in the popcorn itself is linked to causing both lung damage and cancer (http://www.drweil.com/drw/u/QAA400701/Microwave-Popcorn-Threat.html).

4) **Soda pop**. Like processed meats, soda pop has been shown to cause cancer as well. Loaded with sugar, food chemicals, and colorings, soda pop acidifies the body and literally feeds cancer cells. Common soda pop chemicals like caramel color and its derivative 4-methylimidazole (4- MI) have also specifically been linked to causing cancer (https://www.naturalnews.com/031383_caramel_coloring_cola.html).

5) **'Diet' foods, beverages**. Even worse than conventional sugar-sweetened soda pop, though, is "diet" soda pop and various other diet beverages and foods. A recent scientific review issued by the *European Food Safety Authority* (EFSA) of more than 20 separate research studies found that aspartame, one of the most common artificial sweeteners, causes a range of illnesses including birth defects and cancer. Sucralose (Splenda), saccharin and various other artificial sweeteners have also been linked to causing cancer (http://www.dailymail.co.uk).

6) **Refined 'white' flours**. Refined flour is a common ingredient in processed foods, but its excess carbohydrate content is a serious cause for concern. A study published in the journal

Cancer Epidemiology, Mile Markers, and Prevention found that regular consumption of refined carbohydrates was linked to a 220 percent increase in breast cancer among women. High-glycemic foods in general have also been shown to rapidly raise blood sugar levels in the body, which directly feeds cancer cell growth and spread (https://www.naturalnews.com/001812_cancer_prevention.html).

7) **Refined sugars**. The same goes for refined sugars, which tend to rapidly spike insulin levels and feed the growth of cancer cells. Fructose-rich sweeteners like high-fructose corn syrup (HFCS) are particularly offensive, as cancer cells have been shown to quickly and easily metabolize them in order to proliferate. And since cookies, cakes, pies, sodas, juices, sauces, cereals, and many other popular, mostly processed, food items are loaded with HFCS and other refined sugars, this helps explain why cancer rates are on the rise these days (https://www.naturalnews.com/038071_cancer_sugar_sweets.html).

8) **Conventional apples, grapes, and other 'dirty' fruits**. Many people think they are eating healthy when they buy apples, grapes, or strawberries from the store. But unless these fruits are organic or verified to be pesticide-free, they could be a major cancer risk. The *Environmental Working Group* (EWG) found that up to 98 percent of all conventional produce, and particularly the type found on its "dirty" fruits list, is contaminated with cancer-causing pesticides (http://www.ewg.org/foodnews/list/).

9) **Farmed salmon**. Farmed salmon is another high-risk cancer food, according to Dr. David Carpenter, Director of the *Institute for Health and the Environment* at the *University of Albany*. According to his assessment, farmed salmon not only lacks vitamin D, but it is often contaminated with carcinogenic chemicals, PCBs (polychlorinated biphenyls), flame retardants, pesticides, and antibiotics. (http://www.albany.edu/ihe/salmonstudy/pressrelease.html).

10) **Hydrogenated oils**. They are commonly used to preserve processed foods and keep them shelf-stable. But hydrogenated oils alter the structure and flexibility of cell membranes throughout the body, which can lead to a host of debilitating diseases such as cancer. Some manufacturers are phasing out the use of hydrogenated oils and replacing them with palm oil andother safer alternatives, but Trans fats are still widely used in processed foods (https://www.naturalnews.com/010095_hydrogenated_oils_unhealthy.html).

Methods to Reduce Reactive Oxygen Molecules

Primary among these is the production and use of chemicals called antioxidants. Antioxidants inhibit the oxidation of other molecules reducing the levels of reactive oxygen molecules before they can cause damage. Widely used in dietary supplements, antioxidants are classified as either water-soluble or lipid-soluble.

Three most powerful antioxidants and some of the foods that contain them are:

- Beta-carotene – found in colorful fruits and vegetables such as apricots, asparagus, beets,broccoli, cantaloupe, carrots, corn, pumpkin, squash, and watermelon.
- Vitamin C – found in berries, broccoli, Brussels sprouts, cauliflower, honeydew, kale, mango, strawberries, tomatoes, and papaya.
- Vitamin E – found in avocado, chard, mustard or turnip greens, red peppers, sunflower seeds,and nuts.

Other healthy antioxidants include:

- Zinc – found in dairy products, red meat, poultry, beans, nuts, seafood, and oysters.
- Selenium – found in Brazil nuts, tuna, beef, and grain products

Antioxidants slow down or prevent the oxidation of other molecules. By incorporating at least five servings of fruits and vegetables to the diet, the body may be able to lessen the chances of heart disease, neurological disease, cancer and lowered immune system.

Future Directions for Research

Although researchers already have gained substantial insight into the mechanisms and consequences of alcohol–induced oxidative stress, additional studies are required to further clarify how alcohol produces oxidative stress in various tissues. For example, more detailed information is needed on the mechanisms involved in some of the major proposed pathways (e.g., how alcohol–derived NADH leads to ROS production either directly or during the passage of NADH–derived electrons through the mitochondrial respiratory chain). Other mechanisms remain highly controversial, such as the role of CYP2E1 or of various cytokines in alcohol– induced oxidative stress. Additional analyses need to determine the role of alcohol metabolism and its byproducts (e.g., acetaldehyde) in the production of ROS. Finally, it still is unclear how alcohol–induced oxidative stress is produced in tissues where only limited alcohol metabolism occurs.

Many of these issues can be studied using animal models; however, extrapolation of findings from animals to humans will be a difficult task because ROS production and antioxidant status inhumans are affected by numerous nutritional, environmental, and drug influences that are difficult to reproduce in animals. To date, scattered data suggest that the blood of human alcoholics can contain lipids modified by radicals and other reactive molecules as well as immune molecules targeted at such modified lipids and proteins. These data indicate that ROS and other reactive molecules are indeed formed in human alcoholics. (For more information on the presence of such compounds in humans, see the article by Tuma and Casey in this issue.)

Other questions that should be addressed in future research include the following:

1) Do reactive nitrogen species (e.g., nitric oxide) play a role in alcohol–induced oxidative stress in addition to ROS?
2) What is the impact of possible interactions between alcohol and environmental influences such as smoking, use of other drugs or medications, and viral infections (e.g., hepatitis C) on ROS production, oxidative stress, and tissue injury? These interactions must be better defined because most alcoholics are exposed to one or more of these influences in addition to alcohol.
3) How is oxidative stress affected by interactions between alcohol and nutritional factors, such as the levels and specific types of fats ingested? And how much iron is ―safe‖ in a heavy drinker?
4) What are the effects of antioxidants (e.g., vitamin E, vitamin C, or carotenoids) in heavy drinkers? This question is important because some antioxidants can be toxic under certain conditions.

The ability of alcohol to promote oxidative stress and the role of free radicals in alcohol–

induced tissue injury clearly are important areas of research in the alcohol field, particularly because such information may be of major therapeutic significance in attempts to prevent or ameliorate alcohol's toxic effects. As basic information continues to emerge regarding the role of oxidative stress in disease development and the mechanisms underlying ROS–related cellular toxicity, these findings will lead to more rational antioxidant therapeutic approaches. Moreover, these findings could result in the development of more effective and selective new medications capable of blocking the actions of ROS and, consequently, the toxic effects of alcohol.

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