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Review Article

Reactive Oxygen Species in Health and Disease

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During the past decades, it became obvious that reactive oxygen species (ROS) exert a multitude of biological effects covering a wide spectrum that ranges from physiological regulatory functions to damaging alterations participating in the pathogenesis of increasing number of diseases. This review summarizes the key roles played by the ROS in both health and disease. ROS are metabolic products arising from various cells; two cellular organelles are intimately involved in their production and metabolism, namely, the endoplasmic reticulum and the mitochondria. Updates on research that tremendously aided in confirming the fundamental roles of both organelles in redox regulation will be discussed as well. Although not comprehensive, this review will provide brief perspective on some of the current research conducted in this area for better understanding of the ROS actions in various conditions of health and disease.

1. Introduction

The first published article in Pubmed on reactive oxygen species (ROS) dated since 1945; this was retrieved using the keyword "reactive oxygen species." Since then, and at the time this review article was written, the use of ROS as keyword in Pubmed search resulted in more than 117,000 English-written articles, almost 12,000 of which are review articles. Most studies have linked ROS to disease states such as cancer, insulin resistance, diabetes mellitus, cardiovascular diseases, atherosclerosis, and aging, just to list examples. However, numerous articles have also linked ROS to various physiological processes and essential protective mechanisms that the living organisms use for their survival; obvious examples would be their role in immune defense, antibacterial action, vascular tone, and signal transduction. Soon it became clear that in order to maintain a state of homeostasis, living organisms are striving to keep those highly reactive molecules under tight control with the help of an intricate system of antioxidants.

The tremendous amount of research published in this field makes it almost unfeasible for any review article to be comprehensive. Thereby, the main objective of this review is to present recent studies that examine the roles played by ROS in various states of health and disease, with special emphasis on the involvement of two subcellular organelles, the endoplasmic reticulum and mitochondria, in the metabolism of ROS as it relates to these states. Metabolic disorders such as insulin resistance, diabetes mellitus, obesity, and chronic inflammation are focused on. A discussion of several molecules with antioxidant properties is also presented as these might prove to be promising in preventing and/or treating ROS-related diseases. As such, it was not within the scope of this review to deal with all the details.

2. Redox Stress or Redox Regulation?

ROS are highly reactive molecules that originate mainly from the mitochondrial electron transport chain (ETC). Almost all cells and tissues continuously convert a small proportion of molecular oxygen into superoxide anion by the univalent reduction of molecular oxygen in the ETC. The ROS are produced by other pathways as well, including the respiratory burst taking place in activated phagocytes,

ionizing radiation's damaging effect on components of cell membranes, and as byproducts of several cellular enzymes including NADPH oxidases (Nox), xanthine oxidase (XO), and uncoupled endothelial nitric oxide synthase (eNOS) [1].

Due to the potential beneficial role of ROS demonstrated by several lines of research, ranging from their role as signaling molecules [2] to the more unexpected role in improvement of certain cancers [3], the term "redox regulation" might prove to be more accurate than "redox stress"; there have been even some situations where antioxidants are described to be "bad" [4]. However, the term "redox stress" is more commonly used.

3. Examples of ROS Role in Normal Physiological Processes

3.1. Role of ROS in Normal Vascular Diameter Regulation. Mitochondrial ROS, specifically superoxide anion ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2), were demonstrated to play a role in normal vascular physiology in response to such factors as shear-stress [2]. 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 electrobiophysical methods that assessed the ROS generation and the response of vessel diameter to the presence of inhibitors of mitochondrial complexes and antioxidants [2]. 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 whereby 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 [5]. 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 [6–8]. The described model also provides partial explanation for the paradox that whereas moderate ROS levels contribute to regulation of vascular cell function [2], 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.

3.2. 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 O_2 sensor by releasing ROS in response to hypoxia. 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 factor-1 (HIF-1). This has been demonstrated at least in normal (nontransformed) 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 [9], 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 O_2 levels by stimulating angiogenesis, 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 was extensively reviewed by Semenza [10].

3.3. 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 effective, 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 differentiate producing a large progeny of immunological effector cells that are capable of mounting an efficient and antigen-specific immune 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 [1]. The role exerted by ROS in immune responses will be revisited when the inflammasome assembly as a part of chronic inflammation response will be discussed later in this review.

3.4. Role of ROS in Skeletal Muscle Physiology. The skeletal muscle is a target organ for oxidative regulation and/or oxidative stress since it requires a large supply of energy to ensure efficient contraction, and consequently it is liable to be 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 systems control the level of ROS in skeletal muscle, oxidative stress can take place if ROS levels exceed the muscle antioxidant

capabilities, and this can have damaging functional effects [11]. 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 inconsistent depending on the model studied and the experimental design [11–13]. 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 [14]. Others [15] have shown that the elevated levels of antioxidant enzymes activity was also detected in various body organs. Thus, it is thought that exercise, whether acute or chronic, helps in maintaining redox homeostasis since it increases the antioxidant defense mechanisms, and due to the fact that long-term heavy exercise renders both animals and humans more resistant to oxidative damage [14]. 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 [1].

3.5. 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 by Rajendran and colleagues, the posttranslational enzymatic covalent modification of histone and nonhistone 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 cell cycle 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 authors have 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 transcription regulating 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 posttranslation modification of Sirt 1 in the form of desumoylation and hence inactivation of Sirt 1 deacetylation function, and consequently to acetylation and hence activation 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 [16].

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 [17], as well as in the yeast Saccharomyces cerevisiae [18]. 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 (NF-κB). The latter will bind and activate the promoters of various genes. One of those genes is 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 cystine transporter xc2, and manganese SOD (MnSOD) [1, 19].

3.6. "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 the rate 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 tumorigenesis by neutralizing the deleterious effects of ROS [20–23].

Recently, a different 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). Nrf2 is 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, Kelch-like 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

and McMahon [24]. 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 [3]. As Hansson and Libby elegantly described the immune response in atherosclerosis as "double edged sword" [25], 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 with HIF 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 at the 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, diabetes mellitus (DM) and diabetic complications, and CVD, to name only few [26–30].

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 oxidoreductin 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 H_2O_2; 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 amount 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 in apoptosis will be activated [33, 34]. 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 [35]. 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 rate of 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 nonenzymatically formed by the ETC semiubiquinone compound and then enzymatically converted into hydrogen peroxide by superoxide dismutase (SOD). Superoxide anion can also be nonenzymatically 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 enzymes 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 a 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 role in 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) and UCP3 (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 mitofusin 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 [49].

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 in 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.

5. Role of ROS in Metabolic Diseases and Chronic Inflammation

5.1. 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 glyceraldehydes autoxidation, protein kinase C activation, glycation, methyl glyoxal and sorbitol production, hexosamine pathway, and oxidative phosphorylation [50]. Likewise, excess FA leads to peripheral insulin resistance and accumulation of lipid in nonadipose 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 link between 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, it is 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 H_2O_2 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/ob mice), 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 NH_2-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 Forkhead 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 dexamethasone-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, glucokinase. 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 then 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 (K_{ATP}) will be closed allowing the opening of voltage-sensitive Ca^{2+} channels. Increased intracellular Ca^{2+} 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 rho^0 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 [73–75]. The lost response was restored by introducing normal mitochondria into the rho^0 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 β-cells 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 [77].

In patients with T2DM, the form of disease that affects almost 90% of all diabetic patients, some reports 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 of UCPs 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 effect on the retina based on its antioxidant properties 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 [83].

5.4. Role of ROS in Obesity and Obesity-Associated Comorbidities. Obesity—defined as a body mass index of 30 Kg/m^2 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 in activity. 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 elevated risk 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 [84].

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 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 was demonstrated that the ROS produced in the POMC neurons will be accumulating and hence impairing the POMC neurons over time and this is thought to be responsible, at least in part, for our inability to lose weight as we get older. On the other hand, the ROS produced in the NPY/AgRP neurons that are active during negative energy balance will be buffered by UCP2 and 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 antiobesity pharmacological approach attempting to promote satiety or suppress hunger at the hypothalamic level [85, 86].

5.5. Role of ROS in Inflammation and Infection

5.5.1. Role of ROS in Inflammation. Recently, ROS were demonstrated to induce the assembly and activation of inflammasomes, which are multiprotein cytoplasmic complexes involved in mediating cellular inflammation in response to various damaging agents [87–91]. One of the major inflammasomes studied in depth is the NOD-like-receptor- (NLR-) related protein 3 nucleotide-binding domain, leucine-rich repeat containing receptors-related protein 3 (NLRP3) inflammasome, which have been strongly linked to aging and age-related diseases. The mitochondria

are believed to be the main source of inflammasome-activating ROS, although other sources may exist. Excess ROS not only will result in the assembly and activation of the NLRP3 inflammasome but will also inhibit the process of mitophagy, which is a specialized type of autophagy responsible for removal of malfunctioning mitochondria. Therefore, the damaged mitochondria will persist, producing more ROS, and continuing the activation of inflammasome. Alternatively, the cells containing these damaged mitochondria might undergo apoptosis; which is, surprisingly, dependent on ROS as well. Likewise, the voltage-dependent anion channels (VDACs) in the outer mitochondrial membrane are also involved in both inflammation and apoptosis. Although it is so far uncertain what will direct the cell to either chronic inflammation or apoptosis, it is expected that this type of decision is under tight control [92].

In line with the strong association between ROS and chronic inflammation, it was reported that ROS generation correlates with toxicity and pathogenicity of different types of pollutant, such as asbestos and silica particles. In a recent study, Dostert et al. have demonstrated the key role of ROS in mediating the injurious effects of these pollutants that may end in chronic inflammation or even tumor formation. Their findings indicate that upon particles phagocytosis by the immune cells, Nox will be assembled and activated, which will produce ROS in an iron-dependent process [93]. Again, Nox might not be the only source of ROS; other ROS producers may be involved. In any case, the ROS will then activate the inflammasome complex formed of the protein NLRP3, the adaptor ASC, and the substrate procaspase-1. The described stress-related response will end in caspase-1 formation and processing followed by secretion of proinflammatory mediators, including IL-1β and IL-18 [92].

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 effect of ROS is known since the 50s of the last century [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. Affymetrix GeneChips were used to determine the global transcriptional response of *S. aureus* to treatment with subinhibitory 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 involvement in viral infection has also been studied since quite a long time (late 1980s and early 1990s) [96]; more research is still being conducted and producing interesting results, especially in the field of human immunodeficiency 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 co-infected 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].

Key Messages from Section 5. Glucolipotoxicity is associated with both oxidative and ER stress. This is linked to activation of the transcription factor NF-κB and consequently of the proinflammatory gene expression. *In vitro* polyunsaturated FA anti-inflammatory effect is partly mediated by the nuclear receptor PPAR-γ.

TNF-α or glucocorticoids-induced insulin resistance is mediated by excess ROS production, a potential source of which is the sphingolipid ceramide. Excess ROS in turn is thought to work through activation of the JNK signaling pathway that results in differential translocation of two transcription factors; PDX-1, and Foxo1. 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.

ROS induce the assembly and activation of inflammasomes and inhibit mitochondrial autophagy, both processes are related to aging and age-related diseases.

Certain pollutants-induced chronic inflammation or tumor formation is induced by Nox-released ROS-induced activation of inflammasome complex.

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 effect 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 hydroperoxide 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 [100]. 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 effect [100].

Similar to rottlerin, curcumin, which is commonly used as food additive in many parts of the world, exerts anti-inflammatory and antioxidant effect 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 them ethanol and then 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 Cu^{2+}/Zn^{2+} SOD, as well as of catalase. This was reported in the brain tissue of a D-Gal-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 Cu^{2+}/Zn^{2+} 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 [102].

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 [103].

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 C, 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, epoetin δ 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.

Oxidative stress was first induced by treating the cells with glucose oxidase enzyme. The protective antioxidant capability of epoetin δ was then assessed using a commercial oxidative status indicator (2′, 7′-dichlorodihydrofluorescein diacetate; H_2DCFDA). 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 to be involved in the antioxidant renoprotection process [105].

7. 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 offer promising venues 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 [85], 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 Özcan and colleagues have suggested that interventions that regulate the ER stress response offer new opportunities for preventing and treating T2DM [107].

In addition, the serine/threonine kinase, IkappaB 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 [108–111]. Therefore, it is possible that such pathways 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 AP-1 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 has led the authors to conclude that their findings did not corroborate the animal studies results and that AP-1 proved not to be a therapeutic target for progressive human vascular diseases [112].

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.

Key Messages from Sections 6 and 7. In the field of antioxidant therapeutics, ongoing research is being conducted to better understand the mechanism of action of known antioxidant agents and to design and test novel therapeutic agents. As the case in any novel medication testing, systematic approach has to be undertaken utilizing *in vitro* and *in vivo* animal models and human trials; nevertheless, results might not be predictable.

8. Concluding Remarks

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
H_2O_2: 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

ER:	Endoplasmic reticulum
UPR:	Unfolded protein response
mtDNA:	Mitochondrial DNA
T2DM:	Type 2 diabetes mellitus
UCP:	Uncoupling protein
FA:	Fatty acid
NF-κB:	Nuclear transcription factor κB
TLR:	Toll-like receptor
IR:	Insulin resistance
TNF-α:	Tumor-necrosis factor-α
JNK:	c-Jun NH_2-terminal kinase
GSIS:	Glucose-stimulated insulin secretion
POMC:	Pro-opiomelanocortin
NPY/AgRP:	Neuropeptide Y/Agouti-related protein
NLRP3:	NOD-like-receptor- (NLR-) related protein 3 nucleotide-binding domain leucine-rich repeat containing receptors-related protein 3 inflammasome

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