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CALORIC RESTRICTION AND OXIDATIVE STRESS

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6.1 INTRODUCTION

Living organisms create reactive oxygen species (ROS) like superoxide anion, hydroxyl radical, hydrogen peroxide, and nitrogen oxide radical. These short-lived intermediates are deleterious for cells and tissues, and their removal by a scavenger system involving either endogenous or exogenous substances is therefore important. Both processes, ROS generation and inhibition, are well balanced in healthy organisms, but either increased ROS production or insufficient defensive mechanisms create impairment in cell signaling.

ROS may contribute to development of different pathological disorders such as inflammation, diabetes, atherosclerosis, and tumors because ROS play major role in their pathogenesis. In addition, the most important theory concerning pathogenesis of aging is based on oxidative stress as well [1–3]. This hypothesis was formulated on the observation that (a) overexpression of antioxidative enzymes slows down the age-related oxidative damage and extends life span in experimental animals, (b) variations in longevity among species inversely correlate with mitochondrial generation of ROS, and (c) restricted caloric intake decreases levels of oxidative stress, retards the age-related changes, and extends life span in mammals [1]. Overnutrition or caloric restriction significantly affects not only the level of oxidative stress but also cell functions and the life span of living organisms. Elucidation of nutrition-related mechanisms of oxidative stress changes and their cell/tissue consequences may support lifestyle recommendations.

6.2 OXIDATIVE STRESS—BASIC CHARACTERISTICS

In physiological conditions each cell produces short-living intermediates known as ROS. Different intermediates or molecules including both radicals like superoxide, hydroxyl, or peroxyl radicals and reactive molecules like hydrogen peroxide have been identified. They are created from oxygen-containing molecules and free electrons. Superoxide radical may be formed by one-electron reduction of oxygen, whereas two-electron reduction produces hydrogen peroxide. The intermediates are quenched by antioxidant scavenger enzymes with subsequent formation of neutral molecules (e.g., water):

(a) $2\,O_2^{\bullet} + 2H^+ \rightarrow H_2O_2 + O_2$
catalyzed by superoxide dismutase (SOD)

(b) $2\,H_2O_2 \rightarrow 2\,H_2O + O_2$ catalyzed by catalase (CAT)

Hydrogen peroxide has the potential risk to produce hydroxyl radical formed in the presence of transition metals like copper or iron.

(c) $H_2O_2 + Fe^{2+} \rightarrow {}^{\bullet}OH + OH^- + Fe^{3+}$

ROS often initiate chain reaction when electrons are passing to other molecules (saccharides, lipids, proteins, or DNA), by creating their oxidative products (e.g., lipoperoxides). This may have deleterious effects on

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their function, such as gene mutations or inflammation initiating tissue pathology and/or different diseases (diabetes, atherosclerosis, tumors, etc.).

The removal of ROS is therefore important to maintain physiological reactions within the body and to prevent development of pathological processes. The scavenger system is the important counterpart possessing sufficient capacity under normal conditions to quench the ROS intermediates or molecules. Both enzymatic and nonenzymatic systems are present in the body. In addition to enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), or glutathione reductase (GR), there are antioxidant molecules like tocopherol, ascorbic acid, uric acid, bilirubin, etc. possessing antioxidative properties. These are contained in body fluids, and a specific role in oxidative stress has been elucidated in some of them.

ROS formation may be accelerated by internal and external factors. The former include abnormal processes represented by inflammation when released cytokines activate some regulatory molecules [e.g., nuclear factor kappa B (NF-kB)] with consequently created ROS. However, in the most pathological processes the ROS are both a consequence of the cascade events and a promoter of the pathology. Pathogenesis of the disease thus combines different mechanisms including ROS formation that cannot be separated from other reactions because the whole process is highly complex. The power of protective mechanisms represented by scavenger enzymes may significantly determine the level of oxidative stress. On the other hand, external factors like food may influence oxidative stress either by supply of compounds accelerating ROS formation [by advanced glycated end products (AGEs)] or of protective molecules (antioxidant vitamins, polyphenols like resveratrol).

The final status of oxidative stress in the body is rather complicated and dependent on the interrelationship of all endogenous and exogenous influences.

6.3 MITOCHONDRIA—THE MAIN CELL REACTIVE OXYGEN SPECIES GENERATOR

One of the main source of ROS is the respiratory chain in mitochondria in which the electron flux passing complexes I, II, III, and IV is associated with formation of superoxide radical (Fig. 6.1) [4–6]. Under physiological conditions the substrates metabolized in the tricarboxylic acid cycle generate electron donors like NADH and $FADH_2$. The former gives electrons to complex I, the latter to complex II. Then the electron flux continues to coenzyme Q and further to complex III, cytochrome *c*, complex IV, and finally to molecular oxygen with generation of water.

In parallel, the protons are pumped from the mitochondrial matrix into the mitochondrial intermembrane space, generating an electrochemical gradient. This membrane potential with protons outside the inner membrane is a driving force for phosphorylation of ADP to ATP by ATP synthase (complex V), which pumps the protons

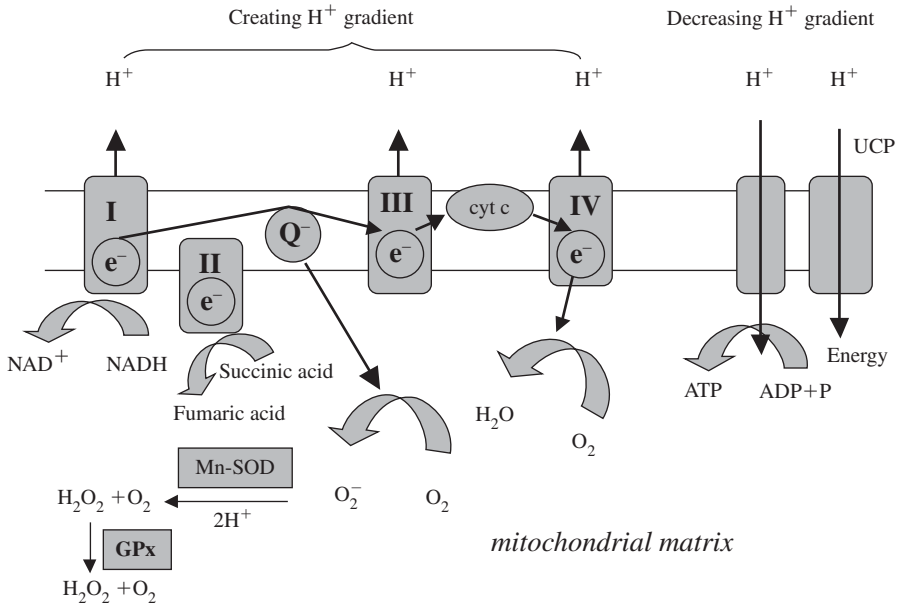

Fig. 6.1 Superoxide radical generation in the respiratory chain of mitochondria: complexes I, II, III, and IV. UCP, uncoupling protein; Mn-SOD, manganese superoxide dismutase; GPx, glutathione peroxidase.

back to the mitochondrial matrix and reduces the membrane potential. Complex V is a rate-limiting step in the generation of ATP in mitochondrial respiration. In addition, ATP generation may be attenuated by proton leak, which shuttles the protons from the intermembrane space to the mitochondrial matrix and thus reduces the number of protons flowing through the ATP synthase. A part of this proton leak is operated by uncoupling proteins (UCPs) regulating the flux of protons through the ATP synthase. The greater level of ATP may inhibit mitochondrial respiration, and therefore uncoupling the proton flux by UCPs is an adaptive mechanism to avoid inhibition of the respiration.

The main source of free radicals is the mitochondrial respiratory chain, where oxygen radical generation has been attributed to complexes I and III. The importance of complex I for ROS production in short-lived compared with long-lived species has been repeatedly documented [7, 8]. The ROS generator was localized within the FeS clusters placed in the hydrophilic matrix domain of the complex I [5, 8], whereas ROS generation within complex III is directed to the cytosolic site [9]. Different localization may explain the finding that mitochondrial DNA (mtDNA) oxidative damage is more common when the ROS are generated by complex I.

The previous hypothesis that ROS production depends on oxygen consumption has not been confirmed. There is clear evidence that mitochondrial membrane potential is a major factor that determines ROS production [10]. Membrane potential may be lowered in the presence of mitochondrial uncouplers or inhibitors. An increased mitochondrial UCP (UCP3) content was accompanied by a lower rate of ROS production [11]. With the use of the UCP2 inhibitor guanosine diphosphate (GDP) an increase in membrane potential and H_2O_2 production has been observed as a consequence of diminished proton leak [12, 13]. Similarly in plant mitochondria, the greater activity of mitochondrial UCPs decreases membrane potential and inhibits formation of ROS at the level of coenzyme Q [14].

The relationship between ROS and UCPs regulating ROS production has been observed [15]. Greater production of superoxide was capable of activating the proton conductance of UCPs and then diminishing superoxide formation as self-mediated feedback [16]. Reactive alkenals created by lipid peroxides have been proposed as the causative factor for UCP activation. Interactions between ROS and UCPs demonstrate a possible mechanism by which free radical concentrations inside the mitochondria may regulate their own production. Any impairment of UCPs decreasing their effectiveness in diminishing membrane potential by increasing proton leak may accelerate superoxide production in mitochondrial matrix [13].

Mitochondrial dysfunction is an important component of aging, type 2 diabetes, neurodegenerative disorders like Alzheimer and Parkinson diseases, and cancers [17, 18]. Accelerated oxidative stress has been considered as the main pathogenic process associated with mitochondrial dysfunction [3]. Superoxide and hydrogen peroxide are two main ROS produced in mitochondria. The majority of mitochondrial superoxide (70–80%) is released to the mitochondrial matrix, whereas the remaining 20–30% is released into the intermembrane space [3]. Intramitochondrial manganese superoxide dismutase (MnSOD) catalyzes the superoxide transformation into hydrogen peroxide inside the mitochondrial matrix. Hydrogen peroxide production is modulated by the mitochondrial metabolic state and by the intramitochondrial concentration of nitric oxide (NO). The rates of hydrogen peroxide production are affected by ion movements through the inner mitochondrial membrane. Its removal is catalyzed in mitochondrial matrix by GPx transforming its molecule into water and oxygen.

NO is produced by NO donors or by nitric oxide synthase (NOS) localized within the mitochondria (mtNOS), although there is still some controversy concerning its existence [3]. The enzymatic reaction requires arginine, $NADPH_2$, and O_2 as substrates and produces citruline, NO, and H_2O (Fig. 6.2). NO inhibits complex III electron transfer and increases superoxide and hydrogen peroxide production. NO is transformed to peroxynitrite, which is a strong oxidant and inhibitor of both complexes I and III. Peroxynitrite remains in the intramitochondrial space and leads to mitochondrial dysfunction and apoptosis. It is the source for nitration of

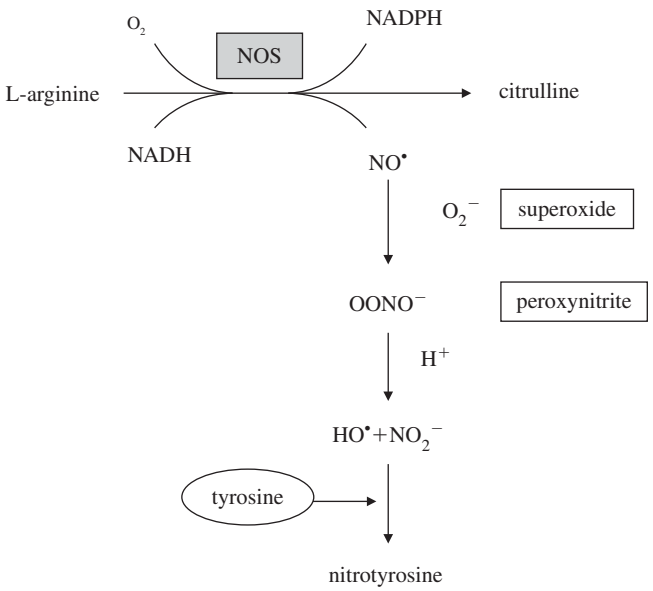

Fig. 6.2 Nitric oxide and nitrotyrosine generation. NOS, nitric oxide synthase.

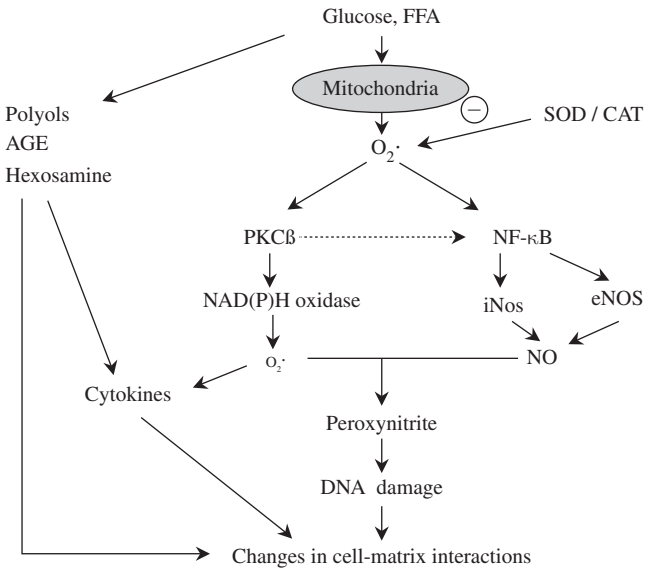

Fig. 6.3 Generation of superoxide by endogenous glucose and free fatty acids (FFA) and relationship to reactive nitrogen species. AGE, advanced glycation end products; SOD, superoxide dismutase; CAT, catalase; PKCβ, protein kinase Cβ; NF-κB, nuclear factor κB; NOS, nitric oxide synthase.

tyrosine residues in proteins and peptides. Nitrotyrosine may be detected as the resulting product.

Oxidative damage is induced by ROS produced primarily as a by-product of mitochondrial oxidative phosphorylation, which is responsible for 85–90% of cellular oxygen consumption [19]. Mitochondrial ROS can cause damage to mtDNA, proteins, and membrane lipids, and thus it contributes to functional and morphological changes observed in pathological states (Fig. 6.3). This process has a self-perpetuating cycle character because increased ROS production leads to incremental damage and further ROS generation [20].

In addition, localization of the ROS production in mitochondria explains why mtDNA is damaged more than nuclear DNA [21]. The rate of oxygen radical attack on mtDNA contributes to differences between long-lived and short-lived animals [22]. A higher rate of oxidative attack in mtDNA was found in short-lived than in long-lived animals, and a similar situation was true with the repair. Oxidative damage of mtDNA may be measured by 8-hydroxy-2′-deoxyguanosine (8-OH-dG), correlating inversely with longevity in birds and mammals [21]. This relationship was not found in the case of nuclear DNA. Mutations in mtDNA caused by ROS are deleterious for cells. The alterations include depressed respiration, enhanced radical formation, increased susceptibility to oxidative stress-triggered apoptosis, accumulation of mutant mitochondria inside the cells, and ROS secretion by mutated cells. These changes observed in short-lived animals prove the role of mtDNA mutations in acceleration of aging [23]. Increased aging rate due to frequent mtDNA mutations was directly demonstrated in mice [24]. Impaired lysosomal degradation of oxidatively damaged mitochondria can also contribute to the aging process [25]. A low rate of mitochondrial ROS production accompanied by low levels of oxidatively damaged mtDNA may therefore delay the aging process. This was demonstrated in different organs including brain, heart, and liver.

Regulation of ROS production is therefore the most important role of mitochondria, thus influencing the aging process and preventing different ROS-dependent disorders [26]. Dietary manipulations involving caloric or methionine restrictions or supplementation with resveratrol may significantly decrease deleterious effects of ROS/reactive nitrogen species (RNS) by improving their homeostasis.

6.4 REACTIVE OXYGEN SPECIES GENERATED BY FOODS

The pandemic of obesity in developed countries has resulted in extensive research of its risk factors and their consequences at the molecular level. A modern lifestyle involving low physical activity as well as overeating characterized by high calorie intake are leading factors in overweight and obesity development. Changes in the ROS production and antioxidant mechanisms have been observed in obese persons, and the possible role of both exogenous factors, physical inactivity and overeating, has been intensively studied. Repeated results obtained in both animal and human studies have demonstrated that physical exercise and decreased food intake confer favorable effects on subjects. The effect of ingested calories on ROS production has been confirmed in experimental and clinical studies (Fig. 6.4).

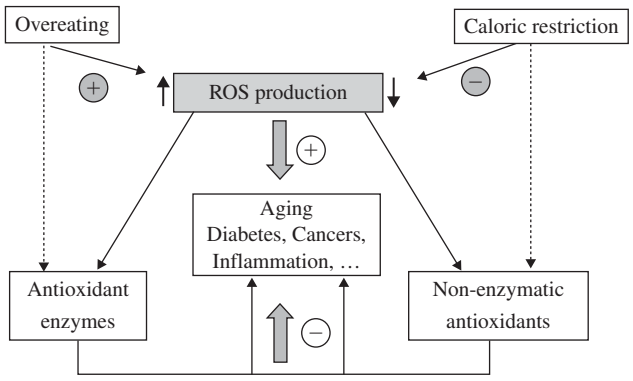

Fig. 6.4 Effect of eating habits on ROS production and its relationship to ROS-mediated diseases.

Higher caloric intake generates more ROS by two mechanisms. First, food that increases body fat, mainly visceral, associated with insulin resistance, subclinical inflammation, and activation of cytokines from adipose tissue causes conditions of greater ROS generation. However, the adipose tissue itself is not the only source of ROS. There are several pathways participating at this process. Adipose tissue resistant to insulin releases more free fatty acids (FFAs) into the bloodstream, which transports them into various organs. The liver, pancreas, heart, vessel walls, and muscles are exposed to the overload of fatty acids, and ectopic fat is then created. More substrates coming into the cells accelerate the Krebs cycle and consequently the supply of NADH and $FADH_2$ into the respiratory chain in mitochondria. FFAs not only cause insulin resistance in the above organs but create more ROS either directly or by activation of the protein kinase C (PKC) rare isoforms (Fig. 6.5) [27]. ROS are closely related to subclinical inflammation by the bridge of activated nuclear factor NF-κB.

Second, food composition and type of preparation may significantly modify the ROS content within the body [28]. Exogenous AGEs produced in greater amounts by broiling and frying of food increase the AGE plasma level and act as contributors of ROS after ingestion of the meal [29] (Fig. 6.6). The AGEs are recognized by specific membrane receptors on macrophages or endothelial cells [receptors for AGEs (RAGEs)], which are then activated to produce cytokines and inflammatory proteins [30]. Alternatively, the AGEs are bound in plasma to soluble receptors (sRAGEs) containing extracellular domain released by

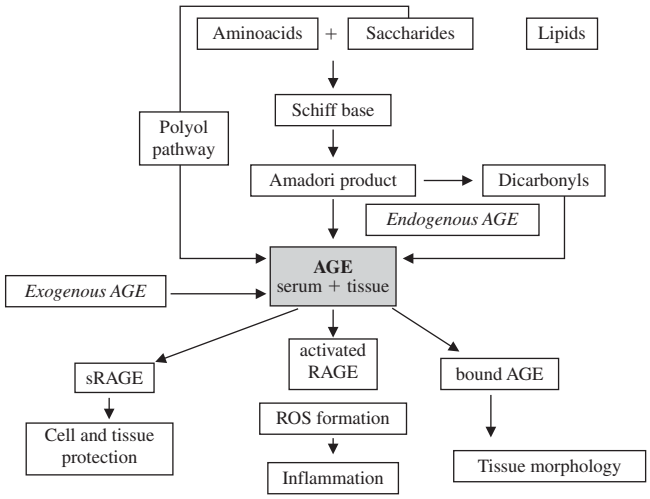

Fig. 6.6 Role of endogenous and exogenous advanced glycation end products (AGE) in ROS formation due to RAGE activation.

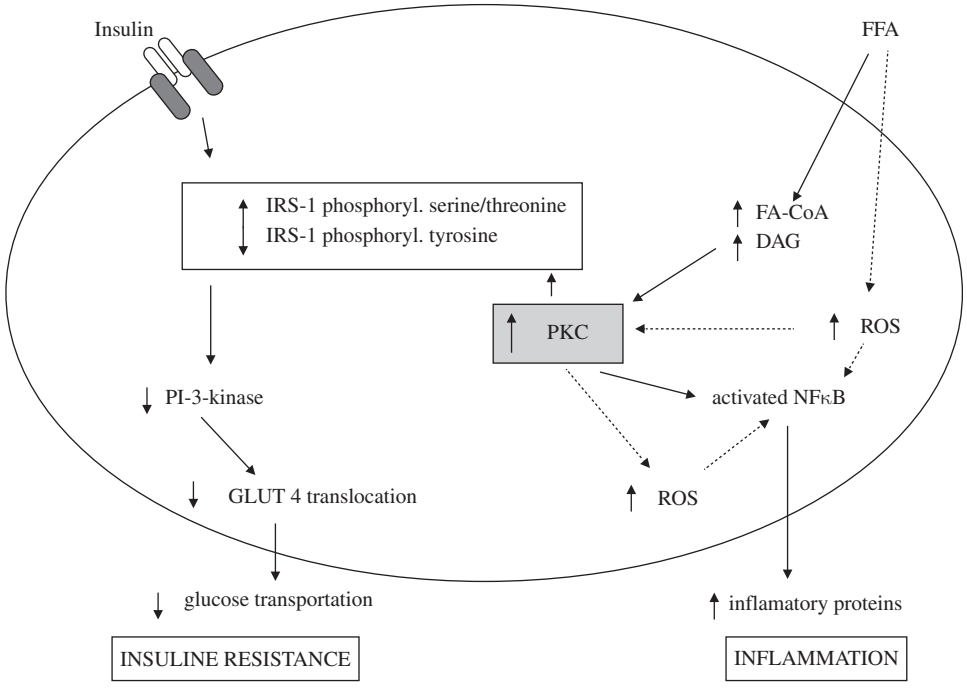

Fig. 6.5 Central role of protein kinase C (PKC) isoforms in the effect of free fatty acids (FFA) on muscle cell ROS production and insulin resistance development. IRS, insulin receptor substrate; PI-3-kinase, phosphatidylinositol 3-kinase; FA-CoA, fatty acid coenzyme A; DAG, diacylglycerol.

metalloproteinases from membrane-bound RAGEs [31]. These complexes circulating in plasma preserve the AGE target cells from activation and prevent both ROS formation and inflammation. Lower sRAGE capacity in individuals with caloric overload may activate target cells to ROS production. Foods containing fats show the highest amount of AGE content [28], and thus they contribute to ROS formation. Acute vascular dysfunction due to AGEs in the food was described in the postprandial state [32]. Excessive AGE consumption represents an independent factor for inappropriate oxidant stress responses, which may promote the premature expression of complex diseases associated with adult life, such as diabetes and cardiovascular disease. ROS may further modify saccharides and lipids by forming glycoxidation products or lipid peroxides that contribute to pathogenesis of atherosclerosis and aging. This process has an autocatalytic character that potentiates the deleterious effect of high saccharide or fat consumption (Fig. 6.7) [33]. Dicarbonyl glycation damage to the mitochondrial proteome may be a preceding event for mitochondrial failure leading to oxidative stress [34].

Some molecular mechanisms associating ROS formation and advanced aging have been recognized. Superoxide and other ROS inside the cell are activators of poly(ADP-ribose) polymerase 1 (PARP1), which is a chromatin-associated nuclear protein acting as a molecular stress sensor [35]. PARP1 enzyme activity is then strongly advanced, and poly(ADP-ribose) may be created. This process consumes NAD^+, which is lacking in a large number of NAD(+)-dependent enzymes, among which sirtuins are more prominent [36]. In addition, PARP1 acts as transcriptional cofactor for NF-kB-dependent gene expression closely associated with inflammation mentioned above.

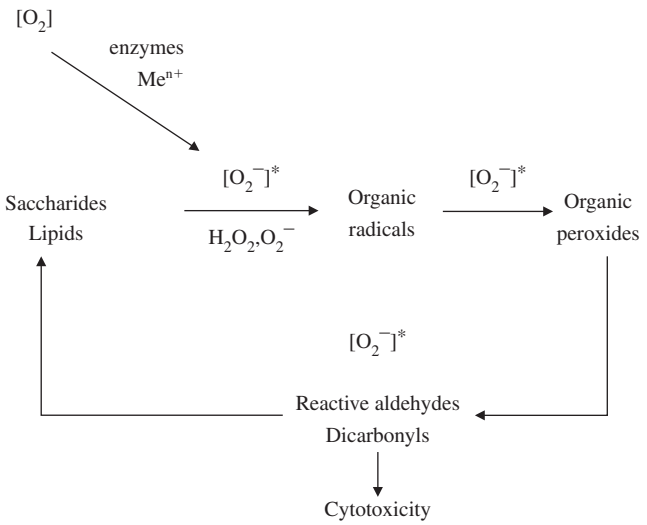

Fig. 6.7 Autocatalytic cycle of oxidative stress.

Caloric overload is therefore a great generator of ROS formation with consequent modification of saccharides, lipids, and proteins. Dietary restriction is the most powerful modulator of the aging process in diverse groups of organisms. Its multifaceted effects are achieved by potentiating the immune responses, lowering oxidative stress, acting as a neuroprotector, and attenuating inflammatory processes [3, 37]. Dietary restriction therefore has robust effects on delaying mortality, increasing life span, and attenuating chronic diseases of old age. It may be important to prevent diseases in older age and to promote healthy aging in humans.

6.5 OXIDATIVE STRESS MODULATED BY CALORIC RESTRICTION AND OTHER FACTORS

A large amount of clinical and experimental data obtained during the last decade demonstrates that overfeeding accelerates ROS production and consequently increases different vascular complications in obese subjects. In addition, it promotes aging rate and lowers longevity. On the other hand, restriction of energy intake was accepted as the main therapeutic principle offering better prognosis to obese persons. Animals under caloric restriction without malnutrition maintain most physiological functions in a youthful state at more advanced ages. Caloric restriction retards age-related diseases such as diabetes, cardiomyopathy, nephropathy, hypertension-related diseases, and tumors [38]. The beneficial effects can be observed when caloric restriction is started both at a young age and in middle age or later [39]. Ongoing studies demonstrate that caloric restriction may reduce the aging rate in rodents [40] as well as in primates [41]. All these promising results motivate investigation to elucidate molecular mechanisms participating in changes of tissues, organs, or organisms as well.

6.5.1 Role of Sirtuins

Genetic and molecular studies demonstrate that low caloric intake is a regulated process with the silent information regulator 2 (Sir 2) gene playing an important role. This is a member of the sirtuin family that mediate different physiological effects and thus influence aging, metabolic diseases, or tumorigenesis [36]. Seven sirtuins (SIRT1–SIRT7) have been described in mammals, belonging to the protein-modifying enzymes known as NAD-dependent histone deacetylases [42–44] that catalyze hydrolysis of acetyllysine [45]. This reaction consumes NAD and releases nicotinamide, *O*-acetyl ADP ribose, and deacetylated substrate. Three sirtuins have been localized in the nucleus, three in

mitochondria, and only one in cytoplasm [46]. Mammalian SIRT1 has several effects in glucose homeostasis [47], insulin secretion [48, 49], and lipid mobilization [50]. A much wider role of sirtuins is suggested in physiological processes including lifespan regulation and cellular response to stress [51].

Reduced energy intake upregulates SIRT1 in muscle, fat, and liver [51]. SIRT1 regulates hepatic glucose metabolism by stimulating gluconeogenesis, which is mediated by peroxisome proliferator-activated receptor (PPAR) coactivator (PGC)-1α at the level of gene transcription [47]. SIRT1 stimulation of gluconeogenesis opposes the insulin effect in the liver promoting gluconeogenesis suppression. Insulin-resistant states are associated with decreased SIRT1 expression and increased expression of UCP2. SIRT1 inhibition may induce adipogenesis by activating PPARγ, whereas glucose signaling and consequently insulin secretion from β-cells in pancreas are reduced. On the other hand, activation of SIRT1 decreases adipocyte formation during osteoblast differentiation from mesenchymal stem cells [52].

Because of the significant role of sirtuins, inhibitors and activators of their expression have been studied. Sirtuin inhibitors may increase p53 activity that stops the formation of tumors and induces apoptosis [53]. Inhibitor will be reliable in the treatment of neurodegenerative Parkinson disease. Sirtuin activators increase life span and cell survival, promote fat mobilization, and increase mitochondrial size and number [54]. Sirtuins link nutrient availability and energy metabolism. Sirtuins, like "molecular sensors," mediate the effects of caloric restriction on the aging process. The most potent activator among natural compounds is resveratrol, a polyphenol antioxidant. Significant reduction of cellular hydrogen peroxide, upregulated MnSOD expression, and increased cellular glutathione content have been observed after resveratrol administration [55]. It is proposed that resveratrol upregulates antioxidant defense mechanisms and attenuates mitochondrial ROS production via sirtuin activation. This effect makes resveratrol a potent neuroprotectant and antiapoptotic agent [56]. Resveratrol acts as a phytoestrogen mimicking the activity of 17β-estradiol and interacting with estrogen receptors α and β (Fig. 6.8; see also section 6.5). Its administration caused significant increase of MnSOD gene expression and augmented its activity several times in human cells [57].

Association of sirtuins with antioxidative defense is proposed by different experimental and human studies [58], although the molecular mechanisms have not been elucidated yet. The relationship between sirtuin activation and SOD transcription has been described [59]. Sirtuins are proposed as mediators of antioxidative mechanisms promoting a decreased level of oxidative stress. A strong association of sirtuin (SIRT1) alterations

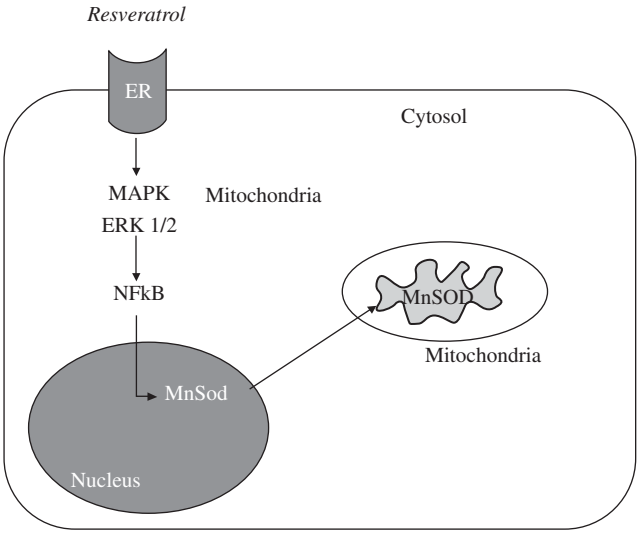

Fig. 6.8 Stimulation of manganese superoxide dismutase (MnSOD) synthesis by resveratrol via estrogen receptor. MAPK, mitogen-activated protein kinase; ERK1/2, extracellular signal regulated kinases 1/2; NF-κB, nuclear factor κB.

with changes in AMP-activated protein kinase (AMPK) has been proposed in an experimental study [60]. SIRT3 has been suggested as an essential player in enhancing the mitochondrial glutathione antioxidant defense system during caloric restriction [61]. SIRT3-dependent mitochondrial adaptation may be a central mechanism retarding aging in mammals [61]. Besides its molecular effects, SIRT1 is a key regulator of vascular endothelial homeostasis controlling angiogenesis, vascular tone, and endothelial dysfunction [62]. A possible link between vascular effects and ROS generation has been suggested.

Sirtuins are potential mediators of caloric restriction and have a promising role as pharmacological targets to delay aging and age-related diseases [63].

6.5.2 Caloric Restriction

Caloric restriction has a protective effect on oxidative damage due to a reduced rate of mitochondrial free radical generation as demonstrated in the rat liver [64], heart [65], skeletal muscle [66], and brain [67]. In many experimental studies 40% reduction in caloric intake was used to demonstrate beneficial effects on reduced ROS production observed already after 7 weeks [68]. Decreased mitochondrial ROS production at complex I of the respiratory chain was found in different studies. However, more detailed studies showed that decreased mitochondrial ROS production is a function of dietary composition. While protein restriction caused a significant decrease in the ROS generation and oxidative damage in mtDNA, no such effects were found after a

carbohydrate- or fat-restricted diet [69–72]. Lowered dietary protein intake and not the calories themselves was responsible for the decreased mitochondrial ROS generation [73]. Special attention was given to methionine restriction, which seems to be responsible for the decreased ROS production observed in a calorie-restricted diet [69, 73, 74]. Methionine supplementation increased mitochondrial ROS generation and percent free radical leak in rat liver mitochondria but not in the heart [75]. Lowering of methionine levels controls oxidative stress in mitochondria by two mechanisms: decreasing the sensitivity of proteins to oxidative damage and lowering the rate of ROS generation [69]. Forty percent calorie reduction may be too large for some persons, and therefore the effects of less restricted diets containing 8.5% and 25% restrictions have been examined. Neither 8.5% nor 25% caloric restriction changed the rates of mitochondrial ROS production or oxygen consumption in the rat liver mitochondria [76]. Opposing effects of a 25% calorie-restricted diet on respiratory chain complex I and III activities have been observed. Decrease in the complex I activity tended to decrease mitochondrial ROS production, whereas increase in the complex III tended to increase ROS generation. It seems that more than 25% caloric reduction is needed to lower the mitochondrial oxygen radical generation due to significantly reduced expression of complex I.

Caloric restriction reduces mitochondrial ROS production and promotes mitochondrial renewal via mitochondrial biogenesis and autophagy [77]. On the other hand, an experimental study in mice showed different results [78]. Caloric restriction increased lipid peroxidation, inflammation, and apoptosis, while decreased mitochondrial bioenergetic efficiency, protein oxidation, and stress response were found. The substantial upregulation of antioxidant enzymes and UCP3 could be a protective response to the heightened oxidative damage in mice. This study illustrates for the first time the detrimental effect of caloric restriction in mice, but no similar data are available in humans. In another study, Santos et al. [79] demonstrated a significant increase of hydrogen peroxide in Goto-Kakizaki and Wistar rats after food deprivation, with different responses in antioxidant enzymes GPx and Gred, which may contribute to oxidative imbalance in rat brain and thus to degeneration and death.

6.5.3 Fasting

Caloric restriction without malnutrition decreases ROS production and brings beneficial effects to subjects concerning life expectancy and delayed onset of several diseases. Although very short fasting has some benefits, prolonged fasting over 24 h may be harmful [80]. Severe food deprivation increases oxidative stress by accelerating mitochondrial free radical generation and by increasing sensitivity of hepatic membranes to oxidative damage (lipid peroxidation). The oxidative changes are induced either by reactive carbonyl compounds or through amino acid oxidation [80]. Starvation induces superoxide anion release from the hepatocytes with parallel decrease of glutathione (GSH) [81]. Depletion of liver antioxidant stores and release of hepatic oxygen free radicals may cause organ damage and increase morbidity in malnourished individuals.

Nevertheless, intermittent fasting has been shown to exert beneficial effects similar to caloric restriction, improving risk factors for cardiovascular disease and increasing life span [82]. Both caloric restriction and intermittent fasting may be related to decreased production of free radicals and improved activity of the mechanisms protecting from damaging agents [83]. In addition, the increased resistance to the oxidative stress found during intermittent fasting could contribute to beneficial effects of this regimen [84]. Every-other-day feeding increased maximum life span in experimental animals by decreasing mitochondrial oxidative stress that is independent from insulin/IGF-1 signaling [85]. The intermittent feeding caused decreased ROS production in complex I but not in complex III of the mitochondrial respiratory chain without changes of oxygen consumption.

Short intermittent fasting has an effect similar to caloric restriction on ROS production in mitochondria, with consequent preventive effect on aging.

6.5.4 Physical Activity

However, energy intake is not the only factor influencing oxidative stress in experimental animals or in human life. Physical exercise was found to influence both ROS production and antioxidant defense mechanisms. It was demonstrated that exercise decreases ROS production evaluated by lowered malondialdehyde concentration [86], increases ROS generation confirmed by higher plasma carbonyl derivatives [87], or has a neutral effect [88]. The antioxidant defense system has been activated as measured by increased SOD activities or plasma vitamin E concentration [86, 87, 89]. However, decreased protective effects of antioxidant mechanisms have been observed, too [90, 91]. These controversial results have been reviewed [92]. Changes in oxidative stress were followed during moderate physical exercise by increased median life span, decreased oxidative damage, and prevention of the decline of cytochrome oxidase activity [93]. Regular exercise seems to retard the accumulation of cell damage and physiological dysfunction [94]. Moderate exercise activates DNA repair and increases resistance against oxidative stress [93]. Positive

effects of moderate exercise have been observed in different organs of experimental animals. On the other hand, high-intensity or long-duration exercise accelerates oxidative stress and decreases GSH-to-GSSH ratio [95–97]. The resulting oxidative stress level is dependent on the exercise intensity. Moderate and chronic physical activity may decrease the oxidative stress level, whereas acute and intense exercise accelerates ROS production and may decrease antioxidant enzyme activities [98, 99].

Caloric restriction in combination with moderate physical exercise may be protective against oxidative stress [100]. Lifestyle modification based on both regimens, lower calorie intake and moderate physical activity, has a beneficial outcome in reduced oxidative stress [101]. Both caloric restriction and physical activity may positively influence mitochondrial function, with consequently increased life expectancy [102]. Negative energy balances induced through either caloric restriction or exercise result in improvements in markers of DNA and RNA damage associated with lowered formation of oxidation products [103].

6.5.5 Sex Differences

The link between sex and cardiovascular disease is well documented in both humans and animal studies [104, 105]. The protection of females against cardiovascular complications is attributed mainly to sex hormones [105], but the role of the mitochondrial respiratory chain has been evaluated in the last few years [106]. Clear sex difference in mitochondrial energy metabolism was observed in the rat liver, skeletal muscle, and adipose tissue [107–109], which could be related to different ROS production in males and females. The sexual dimorphism in liver mitochondrial oxidative capacity was unaffected by caloric restriction in rats, with females showing higher mitochondrial functionality and ROS protection than males [110]. The effect of caloric restriction on ROS generation in cardiac muscle tissue was also compared in female and male rats [106]. Cardiac muscle from female rats exhibits lower mitochondrial content, although more differentiated, without any loss of functionality compared with male rats. Caloric restriction decreases mitochondrial hydrogen peroxide production related to lower activity of the respiratory chain complexes I and III but does not contribute to increased antioxidant activity. A greater mitochondrial differentiation with higher oxidative phosphorylation efficiency in female cardiac muscle has been proposed. However, estrogens may have a positive influence, because increased mitochondrial hydrogen peroxide generation was found in the liver and brain from ovariectomized female rats, whereas hydrogen peroxide production reverted to normal levels when these rats were substituted with estrogens [111]. In another study estrogen-treated rats had antioxidant and hepatoprotective effects [112]. It is of particular interest that both mitochondria and MnSOD are major downstream targets of estradiol signaling [113]. Mitochondria from females were found to contain higher levels of MnSOD than those from males, and their potency to stimulate antioxidant enzymes was higher [114]. However, the effect of estrogen receptor activation to stimulate transcription of mitochondrial MnSOD or GPx is indirect and possibly caused by a signal transduction pathway mediated by MAP kinase, ERK1/2, and NF-κB (Fig. 6.8).

Estrogen appears to have a protective effect for lipoprotein oxidation in postmenopausal supplemented as compared to nonsupplemented women. A sufficient estrogen level may decrease lipid peroxidation. A sex dimorphism was also demonstrated by high-fat diet-induced changes in lipid oxidation and serum activity of paraoxonase 1 (PON1) [115]. Destabilization of the PON1 association to HDL or direct inactivation of PON1 activity accounted for the decreased serum PON1 activities in female rats and thus for its decreased protective effect. Changes in both ROS generation and scavenger enzymes have to be related to sex differences. They could therefore be taken into account when comparing effects of different interventions on oxidative stress in men and women.

6.6 BIOMARKERS INDICATING MOLECULAR CHANGES IN CALORIC RESTRICTION

The level of oxidative stress depends on both ROS production and the effectiveness of the antioxidant system to scavenge it. The actual oxidative stress is thus created in the respective tissue/organ or in body fluids. Biomarkers may characterize the level of oxidative stress as the actual risk of oxidative damage, and they may elucidate the effect of different treatments or interventions.

Direct measurement of ROS production may be used under experimental conditions, but it brings some difficulties. It can hardly be performed in routine clinical practice in humans. Different products of lipid or protein oxidation may be measured more frequently as indicators of oxidative stress. On the other hand, the antioxidant system represented by enzymatic and nonenzymatic compounds may provide information on protective mechanisms decreasing the oxidative stress level. The impaired protective mechanisms may thus exacerbate oxidative stress and induce downstream activation of NF-kB with an inflammation cascade.

The evaluation of oxidative stress in experimental conditions and in humans may therefore offer new

insights in biochemistry and pathophysiology of different diseases.

6.6.1 Reactive Oxygen/Nitrogen Species

Mitochondria is the main ROS generator producing superoxide and hydrogen peroxide as the most important oxygen species [3]. Food or caloric restriction as an effective modulator of oxidative stress may reduce ROS generation in mitochondria, as repeatedly demonstrated in several studies after a restricted diet, and lowered ROS could therefore be anticipated. On the other hand, overfeeding induces more ROS compounds, as confirmed by increased levels of lipid or protein oxidation products.

Hydrogen peroxide is usually produced by electron reduction of superoxide, the reaction catalyzed by SOD. Hydrogen peroxide was found in skeletal muscle in a strain of rats characterized by a self-low caloric intake when they consumed a high-fat diet [116]. Caloric restriction (40%) did not alter proton leak or H_2O_2 production it rat liver [117]. Hydrogen peroxide may be measured as a marker of oxidative stress in experimental conditions, but it cannot be directly measured conveniently in clinical studies.

NO is generated from arginine by mtNOS, and subsequently it may be transformed to peroxynitrite. Hydrogen peroxide is the source of harmful hydroxyl radical, whereas peroxynitrite causes nitration of proteins. Nitrotyrosine as an indicator of protein nitration can be used in studies evaluating the role of NO in the oxidative and/or nitrative stress.

6.6.2 Oxidatively Derived Products

Several markers derived by ROS or RNS and indicating the level of oxidative stress can be determined in tissues or in body fluids. Oxidized plasma lipids or the susceptibility of lipids to in vitro oxidation and conjugated dienes are examples of such oxidative products [92]. Oxidized low-density lipoprotein (LDL) is strongly immunogenic, and autoantibodies produced against oxidized LDL may be used as a biomarker confirming oxidative changes in LDL molecules.

6.6.3 Malondialdehyde and Thiobarbituric Acid-Reactive Substances

Malondialdehyde as a result of lipid peroxidation can be determined spectrophotometrically using thiobarbituric acid (TBA) or by high-performance liquid chromatography (HPLC). Nonspecific reaction products in biological fluids when TBA reacts with other compounds like saccharides or bilirubin are described as TBA-reactive substances (TBARS).

Caloric restriction was associated with decreased plasma oxidized LDL and lowered malondialdehyde concentrations [118]. Similarly, significantly lower urinary malondialdehyde levels have been found after short fasting in healthy women [119]. A nonsignificant decrease in plasma malondialdehyde after 8 days of very low-calorie diet (600 kcal) in type 2 diabetic patients compared with a significantly decreased malondialdehyde concentration in healthy persons supports the suggestion of a role of insulin resistance in reducing the effect of caloric restriction [120]. Similarly, malondialdehyde was unchanged after dietary caloric restriction in streptozotocin-induced diabetic rats, but a small decrease was observed in nondiabetic animals [121]. Chronic undernutrition in marasmic children increased oxidant status and decreased antioxidant mechanisms both associated with increased malondialdehyde concentrations and lower antioxidant potential [122].

In summary, malondialdehyde may be used as a simple oxidative stress biomarker in both clinical and experimental studies demonstrating the effect of dietary regimen. Its plasma concentration may be increased in the acute phase of experimental conditions as a consequence of oxidative stress activation, whereas its concentration decreases with decreased lipid peroxidation. Differentiation between the changes in the acute and chronic phases is needed to properly evaluate the oxidative stress.

6.6.4 Nitrotyrosine

Nitrotyrosine is created from tyrosine residues reacting with peroxynitrite that is generated from NO and superoxide. Caloric restriction has been associated with decreased nitrotyrosine levels in the brain tissue [123, 124]. Nitrotyrosine concentration was lower in skeletal muscle of rhesus monkeys fed a calorie-restricted diet [125]. This study demonstrated that caloric restriction may attenuate the aging process by reducing oxidative stress. In another study, a low-fat complex-carbohydrate diet reduced nitrotyrosine accumulation in comparison with a high-fat sucrose diet in rats [126]. However, limited results concerning dietary effects on nitrotyrosine changes in humans are available, and follow-up studies will be necessary.

6.6.5 F_2-Isoprostanes

F_2-isoprostane is derived from peroxidation of arachidonic acid, and thus it reflects the intensity of lipid peroxidation in biological fluids and tissues. 8-Iso-prostaglandin $F_{2\alpha}$ is a sensitive marker of oxidative stress [127]. Its increased plasma concentration correlated with lipid oxidation in aging of rats [128], whereas caloric restriction

was associated with its decreased plasma as well as liver or kidney concentrations [129]. In addition, F_2-isoprostanes significantly correlate with 8-oxodeoxyguanosine, an indicator of DNA oxidation. Short-term fasting reduces lipid peroxidation products. as demonstrated by decreased urinary 8-isoprostaglandin $F_{2\alpha}$ and malondialdehyde in healthy women [119]. Combination of exercise with either high-calorie or low-calorie diet was associated with significant decrease of serum F_2-isoprostanes [130]. Exercise was considered as the main cause of reduced lipid peroxidation, independent of caloric intake. Lifestyle modification characterized by dietary and exercise intervention may ameliorate factors associated with atherosclerosis. A significantly decreased concentration of 8-isoprostaglandin $F_{2\alpha}$ confirms a reduced ROS production [101].

F_2-isoprostanes as sensitive markers of lipid peroxidation may be used as one of the best indicators of oxidative stress. The F_2-isoprostane concentrations should be assessed simultaneously in plasma and urine because some divergencies bring difficulties in interpretation of data [131].

6.6.6 8-Hydroxydeoxy-guanosine

Mitochondrial DNA damage is related to accelerated ROS production. 8-Hydroxydeoxy-guanosine, a marker of DNA damage due to oxidative stress, correlates with F_2-isoprostanes or malondialdehyde, markers of ROS generation [129]. The association of increased oxidative stress with mtDNA damage was proved in mice heterozygous for the *SOD2* gene encoding mitochondrial MnSOD [132]. In these animals increased 8-hydroxydeoxy-guanosine production was confirmed. Caloric restriction was associated with reduced ROS production and decreased DNA damage as measured by 8-hydroxy-deoxy-guanosine [129]. However, short-term fasting, despite decreased lipid peroxidation, did not reduce 8-hydroxydeoxy-guanosine [119].

Concentration of 8-hydroxydeoxy-guanosine does not always correlate with ROS generation, and DNA damage should be evaluated separately when changes in oxidative stress are observed.

6.6.7 Scavenger Enzymes

Enzymes detoxifying free oxygen radicals and related molecules play an important role in the balance between ROS production and elimination. Enzyme activity significantly determines the intracellular level of oxidative stress; however, the final results frequently depend on cooperation between several enzymes catalyzing a cluster of reactions from ROS intermediates to stable molecules. The oxidative stress evaluation should be based on the estimation of several enzyme activities influencing cascade reactions of intermediate ROS molecules. Genetic and environmental factors may influence enzyme activities and their efficiency to catalyze the appropriate reactions.

6.6.7.1 Superoxide Dismutase The superoxide generated as the most common ROS is removed by two types of superoxide dismutase, cytoplasmic Cu,Zn-SOD and mitochondrial MnSOD. Overeating associated with high energy intake causes dramatic increase of substrates for metabolic pathways, subsequently accelerating the mitochondrial respiratory chain. High superoxide generation is often combined with increased SOD activity. Reduced energy intake produces fewer free radicals, leading to less oxidative damage. It could therefore be interesting to learn whether caloric restriction may influence SOD activity. Experimental studies using calorie-restricted diets showed increased [133], decreased [134], or not changed [121, 135, 136] SOD activity. In another study, MnSOD mRNA was increased after short-term caloric restriction but without elevation of protein level [137]. The increase of myocardial SOD was explained by a synergistic action of olive oil added to a calorie-restricted diet [138]. Short-term very low-calorie diet induced a significant increase of SOD activity in both diabetic and control subjects [120]. Aging was associated with higher ROS generation, but combination with food restriction led to attenuated aging. Similarly, caloric restriction was associated with improved endothelial dysfunction during vascular aging [139]. This effect was caused by an improved ratio of nitric oxide synthase isoforms (iNOS/eNOS) in consequence to reduced oxidative stress. The SOD activity was found to have a key role in this balance because the removal of superoxide may further improve the availability of NO. In another study, the SOD activities, while increased with aging, were attenuated by food restriction [140].

MnSOD as a sole mitochondrial SOD in mammals and birds has a dominant effect on ROS removal in mitochondria. Its expression and activity in the brain correlated with life span in vertebrates, whereas no such relationship was found in the case of cytosolic Cu,ZnSOD or GPx [141]. Hence, MnSOD seems to be an important predictor of the mitochondrial defense system modulating the availability of superoxide for its harmful effects.

6.6.7.2 Catalase Catalase is a ubiquitous heme protein enzyme that catalyzes the dismutation of hydrogen peroxide into water and molecular oxygen. The enzyme is therefore localized in a close downstream position relative to SOD. This enzyme relationship may predict similar changes of their activities [133–135]. Caloric restriction has been associated with increased [133], decreased, or unchanged catalase activities. Elevation of SOD activity sometimes occurs together with

decreased catalase activity [134] when increased GPx activity is observed. An oscillatory and frequently inverse relationship between the activities of catalase and GPx has been proposed [134].

6.6.7.3 Glutathione Peroxidase GPx detoxifies peroxides with reduced form of glutathione (GSH) acting as an electron donor producing GSSG. Different GPx isoenzymes have been described in cytosol or in extracellular space [142]. GPx may share the same substrate, hydrogen peroxide, with catalase. Accelerated ROS production has been associated frequently with increased GPx activity [133, 135]. The enzyme activity was depressed in diabetic animals feeding "ad libitum," but it was increased in both diabetic and control animals by caloric restriction [135]. A significant decrease in GPx activity may suggest either enzyme inactivation by increased ROS or decreased substrate (GSH) availability. In such cases an oscillatory elevation in the catalase activities has been observed [135]. Significantly increased GPx activity together with increased GSH-to-GSSG ratio in kidney was found after caloric restriction in mice [136]. These changes demonstrate increased antioxidant capacity as a consequence of functional impact of dietary regimen.

6.6.7.4 Glutathione Reductase Oxidized glutathione (GSSG) is recycled to reduced form GSH by glutathione reductase (GR), and GSH may be reused in antioxidant defense. GR therefore has a pivotal role in regeneration of GSH. GR and GPx activities were increased in experimental animals after caloric restriction [134]. Such changes increase the capacity of antioxidative system and reduce the level of oxidative stress by caloric restriction. However, other experiments did not demonstrate any changes in liver GR activity in rats affected by 30% to 40% restriction in caloric intake [143, 144]. Improved antioxidative state was associated with changes of other components of oxidative stress (i.e., lowered ROS production and increased activities of other scavenger enzymes).

6.6.7.5 Paraoxonase 1 Paraoxonase 1 (PON1), a sex-dependent enzyme specifically associated to high-density lipoproteins (HDL), has been shown to hydrolyze lipid peroxides present in low-density lipoproteins (LDL). Its activity is sensitive to different diets and other factors as well [145]. Short-term dietary restriction was associated with decreased serum PON1 activity, but no changes were found in liver PON1 mRNA levels [145]. Early and prompt increase in rat serum PON1 activity was induced already during the first hours of fasting [146]. This was followed by progressive decrease of PON1 activity and a profound decrease of liver PON1 mRNA levels. Decreased GPx activity was found during fasting, possibly due to depleted liver glutathione concentration and elevated lipid peroxide levels. Deleterious effect of prolonged fasting is therefore explained by accelerating oxidative stress together with decreased antioxidant mechanisms. Other factors like acidosis or ketone bodies in prolonged fasting may further contribute to decreased PON1 activities by accelerating oxidative stress [147]. Early increase of oxidative stress during the initial hours of fasting may stimulate the antioxidant response of PON1 by increasing its activity [148].

The above results demonstrate that the low-intensity oxidative stress provided by repeated short periods of fasting would elicit a defense response that may enhance protective mechanisms, for example,. antioxidative enzymes [145].

6.6.8 Nonenzymatic Scavengers

The human body contains endogenous compounds that have a protective role and act as antioxidants. Both low (glutathione, ascorbic acid, α-tocopherol, coenzyme Q, lipoic acid, uric acid, bilirubin, etc.)- and high (transferin, ceruloplasmin, albumin, metallothioneins, or chaperones)-molecular-weight compounds have a significant role in maintaining ROS homeostasis. Several compounds have been used in evaluating antioxidative state, such as glutathione, ascorbic acid, etc., but some of them are frequently determined in daily routine practice without any relationship to oxidative stress (uric acid, bilirubin, albumin, etc.).

6.6.8.1 Glutathione Tripeptide glutathione (γ-glutamyl-cysteinyl-glycine) is an important redox buffer inside cells. GSH is required to suppress oxidative stress and to maintain the normal reduced state of the cell. Recycling of reduced (GSH) and oxidized (GSSG) forms is regulated by ROS production and the antioxidant enzymes GPx and GR. The GSH-to-GSSG ratio and antioxidant enzyme activities have been used to assess the oxidative stress level. A lower ratio is associated with accelerated oxidative stress when recycling by GR is diminished. Overfeeding or feeding "ad libitum" in animal experiments decreases GSH concentration, whereas GSSG levels are increased [134, 135]. Caloric restriction is able to increase GSH levels and decrease GSSG; the GSH-to-GSSG ratio is therefore increased. Glutathione redox potential in mitochondria of different organs becomes less negative and/or more prooxidant with aging [149, 150]. Administration of a calorie-restricted diet may attenuate GSH decline and therefore may retard the age-related degenerative processes caused by accelerated oxidative stress.

GSH and GSH/GSSG determination may be used as reliable markers of the oxidative stress. Their correlation

with scavenger enzyme activities offers important information on defense mechanisms against oxidative stress under different conditions in health and disease.

6.6.8.2 Ascorbic Acid Ascorbic acid reduces organic and inorganic radicals. It is involved in regeneration of tocopheryl radical to α-tocopherol, a reaction producing ascorbyl radical as a source of dehydroascorbic acid. Ascorbic acid regeneration has important role in reducing the "pro-oxidative" properties of dehydroascorbic acid. This ambivalent role of ascorbic acid depending on both ROS generation and antioxidative mechanisms may be the source that worsens the oxidative state either inside or outside cells. Regeneration of ascorbic acid and other compounds cycling between the reduced and oxidized forms occurs via the electron transport process (Fig. 6.9).

Ascorbic acid levels may be decreased by aging or generally by accelerated oxidative stress [150]. Caloric restriction attenuating the aging process may also slow down the decline in ascorbic acid level in rat retina. Although short-term very low-calorie diet induced ascorbic acid increase in healthy persons, this was not found in type 2 diabetic patients [120]. Such a difference could be caused by antioxidative abnormalities in diabetes, although data are lacking for more information.

6.6.8.3 α-Tocopherol α-Tocopherol is a lipophilic antioxidative compound preserving membrane against lipid peroxidation. It transforms fatty acid peroxyl radicals to hydroperoxides that are further transformed by GPx. Tocopherol is converted to tocopheryl radical that may be partly reduced by ascorbic acid.

Aging is associated with increased oxidative stress characterized by increased lipid peroxidation markers, protein carbonyls, or nitrotyrosine and lower antioxidant defenses. A positive association was found between plasma SOD or α-tocopherol and survival in a longitudinal study [151]. The highest survival was observed in patients with high serum α-tocopherol and low plasma malondialdehyde concentrations [151]. Caloric restriction attenuates a decline of α-tocopherol content in plasma membrane caused by aging [152, 153]. Restriction of calorie intake was associated with decreased markers of oxidative stress and increased enzyme activities protecting cells against age-related oxidative stress [153]. However, the mitochondrial α-tocopherol content was diminished by calorie-restricted diet in rat [154]. Short-term very low-calorie diet was associated with a decrease of serum α-tocopherol levels, but its content in plasma membranes was not determined [120]. The α-tocopherol changes may contribute to accelerated oxidative stress when production of tocopheryl radicals is not sufficiently reduced. The isolated supplementation by α-tocopherol under conditions with accelerated oxidative stress may induce adverse reactions and further worsen the oxidative state [155].

6.6.8.4 Ubiquinone (Coenzyme Q) Coenzyme Q (ubiquinone) is another lipophilic antioxidant that cooperates with α-tocopherol in plasma membranes. It influences the transmembrane redox system and protects cell membrane against lipid peroxidation. Coenzyme Q has three functions in mitochondria: (a) transfer of reducing equivalents in the electron transport chain (Fig. 6.1), (b) generation of superoxide anion radical, and (c) free radical quenching. As with α-tocopherol, coenzyme Q content declines with aging. Dietary supplementation with coenzyme Q augmented endogenous mitochondrial content in various tissues, but it had no significant effect on the main antioxidant defenses or prooxidant generation [156, 157]. Caloric restriction increased coenzyme Q level and attenuated aging [124, 152, 154].

6.6.8.5 Other Markers The association of fluorescent oxidation products with several indicators of oxidative stress suggests that this measure could be a global marker of oxidative stress [158]. There are no data evaluating these products in caloric restriction. However, fluorescent products highly correlated with oxidative stress associated with smoking.

Different biochemical markers have been used to create the OXY-SCORE index [159], which reflects

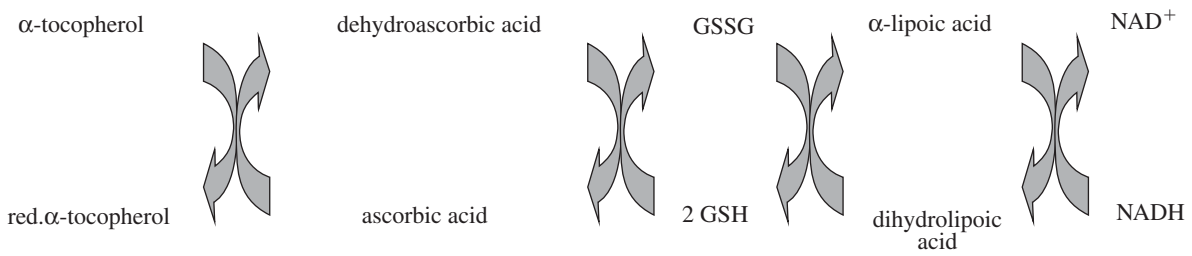

Fig. 6.9 Cycling of antioxidants and their interrelationship.

both oxidant and antioxidant markers. The damage score is characterized by plasma free and total malondialdehyde, glutathione disulfide/reduced form ratio, and urine isoprostanes, whereas the protection score is based on reduced glutathione (GSH), α- and γ-tocopherol levels, and antioxidant capacity. The index calculated as difference between damage and protection scores is related to age and sex, but more data are needed in different conditions.

6.7 CONCLUSION

Oxidative stress is an important process that modifies cellular reactions in both health and disease. It stimulates intracellular pathways promoting development of various diseases but also initiates reparative reactions (e.g., wound healing). Homeostatic mechanisms may sustain oxidative stress under control. Advanced production of ROS or RNS activates protective mechanisms that may eliminate the effects of deleterious compounds when their capacity is sufficient. In the case of insufficient power of these protective mechanisms, homeostasis is impaired.

Increased caloric intake causes overproduction of ROS, especially in the respiratory chain of mitochondria. The substrate overload increases the amount of superoxide generated and then accumulated in the mitochondrial matrix. Insufficient or exhausted capacity of the antioxidant system enables the development of harmful effects in mitochondria and in the cell as well. It may be therefore important to learn whether caloric restriction would be beneficial for ROS homeostasis.

Caloric restriction and prolonged fasting are different conditions. The lower energy consumption or decreased protein (methionine) intake attenuates ROS generation, and thus oxidative products may be decreased. Different changes in antioxidant enzymes involving increased, unchanged, or decreased activities may be dependent on different experimental conditions and on individual responsiveness caused by genetic and epigenetic factors. Increased glutathione (GSH) level may reflect attenuated oxidative stress because greater reducing capacity is present, whereas advanced oxidative stress is frequently associated with decreased GSH and decreased GSH-to-GSSG ratio. The results of oxidative markers depend on timing during the experimental procedure. The initial changes characterized by stimulated ROS production (i.e., increased malondialdehyde concentration) may be followed by depressed ROS generation (decreased malondialdehyde or F_2-isoprostane levels). Short or prolonged exposure to caloric restriction may cause different responses in ROS generation and antioxidative mechanisms. The hormetic role of dietary products increasing cellular stress resistance brings new insights into physiology of oxidative stress regulation [160]. Reduced energy consumption by controlled caloric restriction or intermittent fasting increases life span and protects various tissues against oxidative stress, partly by hormetic mechanisms. Hormesis is based on a biphasic dose-response relationship in which a low dose stimulates and a high dose inhibits protective mechanisms. Thus calorie-restricted diets and their composition may significantly improve ROS homeostasis both in single cells and in the whole body.

On the other hand, prolonged fasting stimulates ROS production and does not provide any benefit. Oxidative products are usually increased, whereas the antioxidant system may be differently modified. More data on molecular mechanisms and regulation of oxidative stress under overnutrition and caloric restriction are still necessary.

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