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Energy Metabolism and Oxidative Stress

Impact on the Metabolic Syndrome and the Aging Process

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Aging can be defined as a progressive decline in the ability of the organism to resist stress, damage, and disease. Although there are currently over 300 theories to explain the aging phenomenon, it is still not well understood why organisms age and why the aging process can vary so much in speed and quality from individual to individual. The oxidative stress hypothesis is one of the prevailing theories of aging. This theory states that free radicals produced during cellular respiration damage lipids, proteins, and DNA thereby accelerating the aging process and increasing disease risk. Under normal conditions, the electron transport chain is the primary producer of the superoxide anion, which is precursor to other highly reactive species such as hydrogen peroxide and the hydroxyl radical. Oxidative stress accumulates when prooxidants overwhelm the antioxidant defense mechanisms. This is dependent on a number of factors including free radical production, susceptibility of tissue to stress, and strength of the defense and repair system. Oxidative stress has been implicated in a number of chronic disease states usually grouped under the umbrella of the metabolic syndrome and is thought to contribute to the aging process. It has been hypothesized that the production of free radicals is dependent on resting metabolic rate and this may have an impact on the aging process. However, other factors, such as mitochondrial function, may be important in the production of free radicals and the subsequent effect on aging and disease states.

Key Words: Oxidative stress; aging; obesity; type 2 diabetes; energy expenditure.

Introduction

Aging can be defined as a progressive decline in the ability of the organism to resist stress, damage, and disease *(1)*. It is characterized by an increase in the incidence of degenerative disorders including cancer, obesity, cardiovascular disease, decreased immune function, and type 2 diabetes *(2)*. The aging process proceeds at varied rates in different individuals. Although there are currently over 300 theories of aging, it is still not well understood why organisms age and why the biological aging process can be so variable from individual to individual *(3)*. In this review we will present the free radical/oxidative stress theory of aging, discuss how free radicals, particularly reactive oxygen species (ROS), are formed and how they damage lipids, proteins, and DNA. We will also discuss how oxidative stress is involved in the progression of chronic diseases including two facets of the metabolic syndrome, obesity and type 2 diabetes mellitus. Finally, we will examine the connections between energy expenditure, ROS production, and oxidative stress and examine how these factors are affected by obesity.

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The Free Radical/Oxidative Stress Theory of Aging

The oxidative stress hypothesis is one of the prevailing theories of aging. This theory states that free radicals produced during cellular respiration damage lipids, proteins, and DNA accelerating the aging process and increasing disease risk *(4)*. The hypothesis is supported by numerous observations. For example, maximal lifespan is inversely related to the amount of free radical production *(5)* and levels of oxidatively damaged molecules have been shown to increase with age *(6–8)*. Furthermore, manipulations such as calorie restriction or overexpression of antioxidant genes reduce the levels of tissue oxidative damage in parallel to increased lifespan *(9–11)*.

Reactive oxygen species are a family of free radicals including superoxide ($O_2^{\bullet -}$), hydroxyl ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2) that are produced as a result of reactions with oxygen. Under normal conditions, the electron transport chain is the primary producer of the superoxide anion *(12)*. The electron transport chain is responsible for the transport of electrons through complexes in the chain creating a proton gradient. This proton gradient produces the energy needed to generate ATP. Figure 1, adapted from Batandier et al. *(13)* and David R. Caprette (caprette@rice.edu;2000), illustrates the electron transport chain and the potential sites for free radical production. Electrons leak

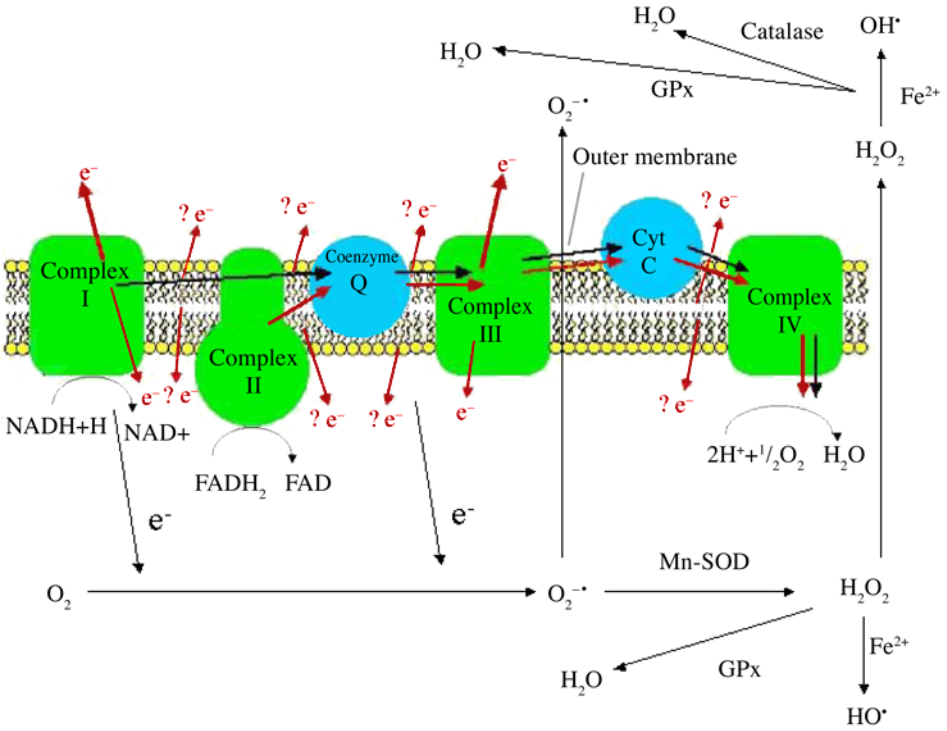

Fig. 1. Electrons leak while they are being transferred along the complexes of the electron transport chain. Leaked electrons can react with molecular oxygen to form superoxide. Superoxide can react with Mn-SOD, either in the mitochondrial matrix or within the intermembrane space to form H_2O_2. Depending on the conditions, H_2O_2 is either broken down by catalase of glutathione peroxidase to form H_2O or can react with metals to form the highly reactive $OH^{\bullet}$ radical. Figure adapted from Batandier et al. *(13)* and Caprette, D. R. (Caprette@rice.edu; 2000)

during their transfer from NADH dehydrogenase in Complex I to ubiquinone. Using bovine heart mitochondria, Turrens et al. *(12)* observed that the inhibition of electron transport by rotenone or antimycin A resulted in an increase in $O_2^{\bullet -}$ production from reduction of NADH dehydrogenase. Leakage can also occur during transfer within Complex III, particularly at the point of cytochrome b *(14)*. Measurements of oxygen consumption and free radical production in isolated mitochondria from heart and brain tissue of rats indicate that when ADP is not present (state 4 respiration), the addition of succinate results in the production of H_2O_2 *(15)*. It was estimated that during state 4 respiration isolated mitochondria generate 0.6–1.0 nmol of H_2O_2/min/mg protein *(16)*, which accounts for approx 2% of total oxygen uptake. However, these earlier studies were performed under artificial conditions, and subsequent studies under more physiological conditions have reduced this value to 0.2% *(17)*. In addition, Herraro and Barja *(18)* demonstrated that when either malate or pyruvate are used as substrates, there is no difference in H_2O_2 production whether ADP is present or not indicating that free radical production is possible in both state 3 or 4 respiration. There are a number of other ways ROS can be produced: (1) during peroxisomal β-oxidation of fatty acids, which generates H_2O_2 as a byproduct *(19)*; (2) during metabolism of xenobiotic compounds by microsomal cytochrome P-450 enzymes *(20)*; and (3) when phagocytic cells attack pathogens with a mixture of oxidants and free radicals including $O_2^{\bullet -}$, H_2O_2, nitric oxide ($NO^{\bullet}$), and hypochlorite *(21)*.

Oxidative Damage to Biomolecules

Oxidative stress was first defined in 1991 as "a disturbance in the prooxidant–antioxidant balance in favor of the former, leading to potential damage" *(22)*. There is a delicate balance between free radical production and the antioxidant defense mechanism as illustrated in Fig. 2 modified from Schulz et al. *(23)*. Oxidative stress can result from either an increase in ROS production, or a reduction in antioxidants *(24)*. Whichever the cause, oxidative stress results in damage to lipids, proteins, and DNA.

Lipids

Lipids are the most susceptible to free radical attack. Bielski et al. *(25)* demonstrated that perhydroxyl radical (the conjugate acid of superoxide) can react with unsaturated fatty acids in the lipid membrane leading to damage of the membrane. Hydroxyl radicals or other highly reactive oxidants can also react with lipids in the membrane by removing a hydrogen atom generating a carbon-centered radical that can rapidly combine with oxygen to form a peroxyl radical. Wagner et al. *(26)* demonstrated that lipid peroxidation was exponentially related to degree of saturation of fatty acids. Peroxyl radicals are then capable of removing hydrogen atoms from other fatty acids. This results in

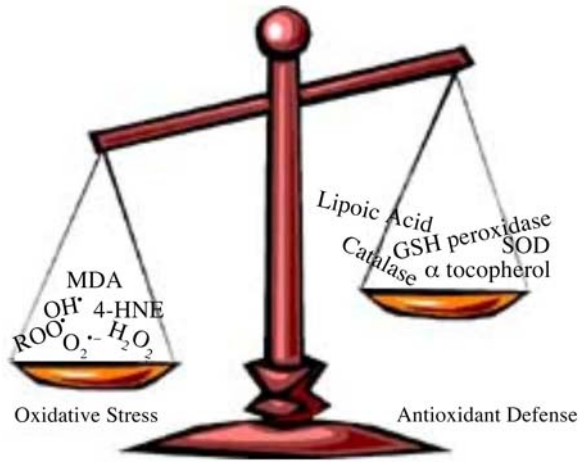

Fig. 2. The fate of a cell is determined by the balance between production of free radicals from endogenous and exogenous sources and the protection of tissues against these sources by antioxidative defense mechanisms. Figure adapted from Shulz et al. *(23)*. MDA, malondialdehyde; OH•, hydroxyl radical; 4-HNE, 4-hydroxy-nonenal; H_2O_2, hydrogen peroxide; $O_2^{\bullet}$, superoxide radical; ROO•, peroxyl radical; SOD, superoxide dismutase; GSH peroxidase, glutathione peroxidase.

a cycle of lipid peroxidation resulting in the formation of hydroperoxides *(27)*. In addition, products of lipid peroxidation such as malondialdehyde (MDA) can also react with DNA bases and introduce mutagenic lesions *(28)*.

Proteins

Superoxide and H_2O_2 have little effect on proteins. However, OH• and singlet O_2 can react with proteins to generate a number of products such as amino acid peroxides and protein carbonyls. Because proteins are constantly turning over, it was assumed that accumulation of damaged proteins would be unlikely *(29)*. In addition, many proteins can sustain considerable damage without functional consequences *(24)*. However, the ability to degrade and remove damaged proteins appears to decline with age *(29)*. High-molecular-weight proteins are more susceptible to oxidation than others. Yan et al. *(30)* demonstrated that aconitase, an enzyme in the citric acid cycle, is particularly susceptible to oxidation resulting in the production of carbonyl derivatives and a 62% decrease in enzyme activity in flies from 7 to 15 d of age. In addition, adenine nucleotide translocase (ANT) is also susceptible to oxidation and the age-related increase in ANT carbonyl content correlated with a loss in functional activity of flight muscles *(31)*. Thus, it is apparent that oxidative damage to proteins can affect the function of important systems and may thereby regulate the aging process.

DNA

Even if oxygen species lead to damage to lipids and proteins, ROS-induced damage to DNA may have the most significant impact on aging *(32)*. Although DNA is generally very stable, it can undergo spontaneous fragmentation over a lifetime. Although superoxide and hydrogen peroxide at physiological levels do not react with DNA or RNA, the hydroxyl radical will react with sugars, purines, and pyrimidines of the genetic material. For example, OH• can add on to carbons at positions 4, 5, and 8 in the purine ring of adenine or guanine. OH• can also react with pyrimidines to produce a number of byproducts including thymine glycol or dihydroxycytosines. Furthermore, removal of hydrogens from the deoxyribose sugars by free radicals produces a number of carbon-centered radicals, which rapidly convert to sugar peroxyl radicals *(24)*.

Mitochondrial DNA (mtDNA) may be more susceptible to damage than nuclear DNA (nDNA) due to (a) the close proximity to the site of mitochondrial free radical production; (b) reduced protective covering of histones; and (c) inefficient repair mechanisms *(33)*. This is supported by data from Hamilton et al. *(34)* demonstrating that 8-oxo-2-deoxyguanosine (oxo8dG) generation in mtDNA isolated from liver, heart, and brain were 6-, 16-, and 23-fold higher than in nuclear DNA from these tissues. In addition, calorie restriction in mice results in a decrease in damage to mtDNA with no change in nDNA *(35)*. MtDNA encodes for 37 genes including 13 subunits of the electron transport chain *(36)*. Oxidative damage to mtDNA can set in motion a vicious cycle of mitochondrial impairment and reduced oxidative capacity *(36)*. In fact, the most common mtDNA deletion accounting for 30–50% of all deletions in skeletal muscle is mtNDA^{4977}, which contains a portion of the mitochondrial genome that encodes for 7 of the 13 proteins involved in respiratory chain function *(37,38)*. Therefore, damage to mtDNA by oxidative stress may not only lead to further damage but may also cause impaired cell functioning.

Oxidative Stress and Disease

Oxidative stress has been implicated in the progression of a number of disease states such as type 2 diabetes and cardiovascular disease and is thought to contribute to the aging process (Fig. 3). Furthermore, it is independently associated with obesity, hyperlipidemia, hyperglycemia, and hypertension *(39–44)*. ROS serve as precursors to the formation of oxidized low-density lipoproteins (oxLDLs), essential to the formation of atherosclerotic lesions *(45)*. The presence of ROS also results in decreased expression of adiponectin and increased expression of pro-inflammatory cytokines including tumor necrosis factor (TNFα), plasma activator inhibitor (PAI 1), and interleukin 6 (IL-6) *(46)*. Block et al. *(47)* observed a positive relationship between isoprostane concentration and C-reactive protein, the acute phase reactant responsible for a number of proatherogenic actions including increasing cell adhesion molecules and PAI 1 activity *(48)*. Oxidative stress is also implicated in the development of insulin resistance and type 2 diabetes *(49)*. Lipid peroxidation is higher in obese men when compared to lean

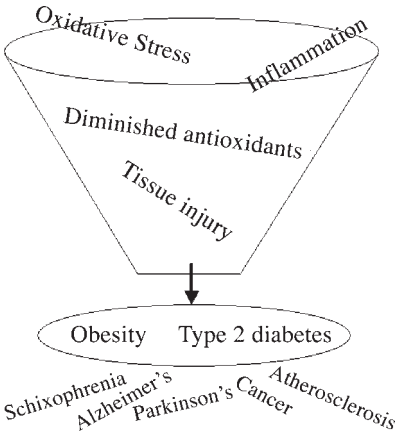

Fig. 3. Oxidative stress is involved in the progression of a number of disease states through increased oxidative stress, diminished antioxidant activity, increased inflammation, and tissue injury.

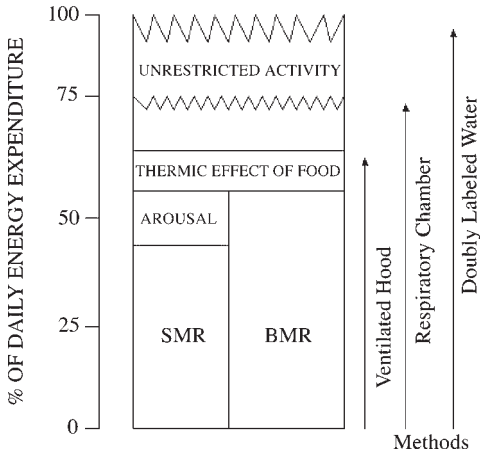

Fig. 4. Total energy expenditure is divided into (1) basal metabolic rate, which is the sleeping metabolic rate and the energy cost of arousal, (2) thermogenesis, the response to a stimulus of which diet induced thermogenesis is the most significant, and (3) energy expended in physical activity, either structured or spontaneous physical activity. Figure adapted from Ravussin *(56)*.

and is positively associated with fasting glucose *(49)*. Individuals affected by metabolic syndrome have elevated isoprostane levels and attenuated antioxidative capacity of small, dense, HDL subfractions, known to be protective against systemic oxidative stress *(50)*. Finally, obesity is associated with increased oxidative stress and oxidative stress may be the link between the obese state and related diseases *(46)*. Oxidative stress has also been implicated in a number of neurological disorders including Alzheimer's, Parkinson's, and schizophrenia *(51–53)*.

Total Energy Expenditure

There are a number of factors that are thought to contribute to an individuals "oxidative status" including sex, body composition, age, smoking status, diet, and external environment. In addition, since ROS production is a byproduct of cellular respiration, it has been suggested that energy expenditure may have an impact on oxidative stress. Total energy expenditure (TEE) can be divided into three main components (Fig. 4). Basal (BMR) or resting metabolic rate (RMR) is the minimum energy required to maintain the integrated systems of the body operational and to maintain body temperature *(54)*. BMR is the energy expended by a subject resting in a relaxed state in the morning following a 12-h fast under comfortable, ambient conditions. It is the sum of the sleeping metabolic rate (SMR) and the energy cost of arousal. RMR accounts for approx 50–70% of total daily energy expenditure *(55)* . The second component of TEE is thermogenesis, which is defined as the increase in metabolic rate in response to a stimulus such as food, cold or heat, psychological influences, or in response to drug administration. The most common form of thermogenesis is in response to food intake. Meal-induced thermogenesis accounts for approx 10% of TEE *(56)*. Finally, physical activity level is the most variable component of energy expenditure. It can be divided into two main components: energy spent for exercise and energy expended on activity that is not exercise, i.e., spontaneous physical activity also known as non-exercise activity thermogenesis (NEAT) *(57, 58)*. Physical activity can account for as little as 20% in sedentary individuals to 50% in very active individuals *(59)*.

Energy Expenditure and Oxidative Stress

Pearl's (1928) rate of living theory states that the duration of life among species varies inversely with the rate of energy expenditure *(60)*. At the time, the mechanisms relating energy expenditure to the aging process were unknown. However, in 1956, Harman *(4)* postulated that free radicals produced during normal cellular metabolism can damage cell constituents leading to abnormal cell function and eventually to cell death. This theory provided for the first time a direct link between energy metabolism, aging, and lifespan. Earlier studies demonstrated a positive relationship between free radical production and species-specific metabolic rate *(61,62)* and an inverse relationship with both of these to maximum lifespan *(63)*. On the contrary, Speakman demonstrated in mice a positive association between absolute or adjusted metabolic rate and maximal lifespan *(64)*. This same pattern has also been observed in dogs and flies *(65,66)*. In addition, obesity, which is associated with shorter lifespan, is characterized by high absolute metabolic rate but often normal or even low when adjusted for body composition *(67,68)*. In most cases obesity is associated with low levels of physical activity, although this has not always been confirmed *(69)*. Obesity, however, is associated with an increase in oxidative stress *(46)* and the fact that obese individuals have a relatively normal metabolic rate for their body size does not support the idea of a direct relationship

between metabolic rate and oxidative stress. Other factors may be significantly more important to aging and longevity than resting metabolic rate.

Mitochondrial Function and Aging: Similarity to Obesity and Type 2 Diabetes

The lack of a consistent relationship between energy expenditure and longevity may indicate that factors other than total oxygen consumption may be more important in determining the rate of aging. The accumulation of oxidative damage associated with aging may be more a function of damaged, "leaky" mitochondria producing more free radicals than of total oxygen consumption per se. It is however not yet clear whether it is the production of free radicals or overall mitochondrial dysfunction that initiates this "vicious cycle." In support of mitochondrial dysfunction as a primary cause, Desai et al. *(70)* observed a decline in enzyme activity in complexes I, III, and IV in aged mice compared to young. Similar declines are also seen in humans *(71,72)*. This is supported by studies using NMR, which observed a 50% decline in ATP production in the quadriceps muscle of old individuals when compared to young *(73,74)*. Alterations in the complexes of electron transport that result in reduced binding affinity may provide a mechanism for increased production of free radicals in aged animals *(70)* supporting this "vicious cycle" hypothesis. This pattern is also evident in other states of increased oxidative stress including obesity and type 2 diabetes mellitus. Skeletal muscle from obese non-diabetic and type 2 diabetic subjects have reduced oxidative capacity *(75)*. This reduced oxidative capacity can result in an elevation of intramyocellular triglyceride content *(76)* and the accumulation of fatty acids and fat metabolites around the mitochondrial matrix. Schrauwen proposed that, in obesity, the accumulation of fatty acids in and around the mitochondria where oxidative processes take place and ROS are produced *(77)* makes them prone to lipid peroxidation which may eventually result in damaged mitochondria and reduced oxidative capacity *(77)* as seen in type 2 diabetes and aging *(73,78)*. This is consistent with data demonstrating an increase in lipid peroxidation in obese individuals *(40,46)* and its association with impaired insulin sensitivity *(79)*. Mitochondria are normally protected against the effects of ROS by a complex system of antioxidants and scavenging enzymes including superoxide dismutase (SOD), catalases, glutathione reductase, and glutathione peroxidase. Diabetic patients have been shown to have reduced expression of SOD and glutathione reductase *(80)*. With age, the percentage of oxygen that is converted to ROS increases *(81,82)* and this detrimental process is amplified by reduced natural defenses, i.e., SOD. Moreover, increased ROS levels cause mutations in mitochondrial DNA *(83)*, further impairing mitochondrial oxidative capacity. Collectively, impaired mitochondrial oxidative capacity, coupled with reduced mitochondrial biogenesis, and impaired ROS defense system would further exacerbate the storage of lipids inside the muscle cell further perturbing mitochondrial structure and function. It is apparent that in obesity and type 2 diabetes, increased oxidative damage and reduced oxidative capacity can result in a rapid deterioration in mitochondrial function that is also associated with aging. Furthermore, this mechanism implicating mitochondrial impairment in the accumulation of oxidative damage provides a direct connection between the electron transport chain to the accumulation of oxidative stress in aging and disease states and helps to explain the connection between obesity and the development of disease.

Conclusion

In summary, production of free radicals and the resulting oxidative stress are a part of normal energy metabolism and seem to have a significant impact on aging and disease. While it was originally thought that total oxygen consumption and metabolic rate may have a linear relationship with free radical production, recent data indicate that this relationship is not as simple as was once thought. Recent research has emphasized the importance of mitochondrial dysfunction in the development of diseases of the metabolic syndrome and in the aging process. The health of the mitochondria may provide a more logical link between oxidative stress, facets of the metabolic syndrome, and the aging process as well as why some individuals are more susceptible to the accumulation of oxidative stress than others.

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