MINIREVIEW

How Diet Influences the Aging Process of the Rat (43684)

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Abstract. **Aging is a time-dependent, deleterious process known to be influenced by multiple factors and processes. Recent gerontological investigations have clearly shown nutrition to be the most powerful extrinsic factor influencing the aging process. That role has been clarified by recent inquiries on such underfeeding paradigms as calorie restriction. The idea of reduced calorie intake as a potent aging modulator contrasts dramatically with the traditional view that "more is better" which springs from the consideration of nutrition only as a source of energy and nutrients required to maintain health. This review presents pertinent data on how nutrition may influence the longevity of organisms and modulate age-related diseases.** [P.S.E.B.M. 1994, Vol 205]

Biological aging can be characterized by a breakdown and loss of coordination between various control and integrating systems (1) leading to disturbances in the homeostasis of cells and organs, and loss of the organism's adaptability to meet both internal and external challenges (2). Recurrent observation over the years has shown that the long-term nutritional status of organisms plays a crucial role in the maintenance of cellular homeostasis that protects against age-related deterioration.

Because of health and disease concerns related to nutritional deficiency, the emphasis of nutritional research tended in the past to concentrate on malnutrition or undernourishment. This traditional approach became the foundation of nutritional sciences and served that purpose well. However, in modern societies where foods are readily available due to advanced technologies in food production, serious nutritional deficiencies are no longer commonly a great threat to health. In fact, it is increasingly evident that overnutrition due to calorie consumption that exceeds energy expenditure now underlies a major problem in modern medicine and public health. Examples of the adverse effects of overnutrition are evident in such major degenerative conditions as obesity, cancer, diabetes, and atherosclerosis. Because these overnutrition-related problems exacerbate aging processes (3), it is important to view nutrition as the most effective interventive measure we have with which to reexamine the aging process and pathogenesis (2, 4).

Over the last two decades, intensive research exploring nutrition in animal aging has shown that excessive calorie intake over a lifetime seems to accelerate the age-related decline seen in physiological dysfunctions. Indeed in rodents, excess calorie intake does not seem solely related to obesity but to the mechanisms of the aging process and longevity as well. Published data clearly point out that interactions of nutrition between biological aging and disease are very much interdigitated, as schematically presented in Figure 1. While continuous attention to nutritional adequacy to promote growth and development is needed, we also need to recognize the life-long, long-term effect of overnutrition on the biological aging process.

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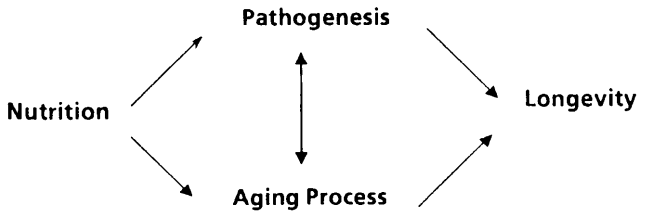

Figure 1. Interrelationship between nutrition, pathogenesis, aging, and longevity.

Methods Used for Life Span Extension

To appreciate the impact of nutrition on the aging process, it is worth presenting a brief background on the methods considered useful for life span extension. Extending life span is one of the strongest of human instincts. Historical records abound with interesting but bizarre accounts of our progenitors' attempts to accomplish longevity. Examples include transfusion of young blood, removal of large intestines, and transplantation of testicles of young monkeys (5). Yet there is very little scientific validity in any claim of the success of these endeavors.

Armed with a better understanding of the biological aging process, modern gerontologists are carrying out experiments to intervene in the aging process. Some of these scientific trials and examples are listed in Table I (5–22). As shown in the table, various experimental approaches have been tried to retard the aging process using different models ranging from lower organisms, such as medflies and nematodes, to nonhuman primates and humans. These experimental interventions fall into five categories: physical modification of organisms, pharmacological drug treatment, hormonal therapy, nutritional interventions, and genetic modification of lower organisms.

Table I. Experimental Interventions on the Aging Process

	Experimental model	References
1. Physical modification		
a. Growth retardation	Rodents	4
b. Lowering body temperature	Poikilotherms	6
c. Physical exercise	Rodents, humans	7
d. Hypophysectomy	Rats	8
2. Drug treatment		
a. Gerovital	Humans	9
b. Centrophenoxine	Humans	10
c. Deprenyl	Rodents, humans	11
d. L-Dopa	Humans	12
e. Melatonin	Rodents	13
3. Hormonal therapy		
a. Growth hormone	Humans	14
b. Testosterone	Humans	15
c. Dehydroepiandrosterone (DHEA)	Rodents, humans	16
4. Nutritional intervention		
a. Dietary components	Rodents	17
b. Dietary supplementation	Rodents	18
c. Calorie restriction	Rodents, monkeys	19–21
5. Genetic modification		
a. Gene identification	Lower organisms	22

The extent and success of these experimental interventions varied greatly among modulated parameters measured, depending on the specificity of the intervention on given model systems and what indices were used to measure the extent of intervention. Although signs of promise are seen in some interventive experimentations, further systematic investigations are needed because some of the results are weakened by lack of experimental reproducibility. One outstanding exception is nutritional manipulation of laboratory rodents by calorie restriction. Calorie restriction has emerged as the most beneficial antiaging intervention in rodents and other lower organisms (2, 3). Because of the well-established acceptance of calorie reduction as a model for aging studies, this presentation will focus on experimental data that highlight how such a relatively simple intervention can indeed modulate the aging processes.

Calorie Restriction as an Antiaging Measure

As a laboratory practice, implementation of calorie restriction was usually accomplished by 40% reduction of the amount of *ad libitum* intake of a well-balanced chow (19). Thus, calorie intake was reduced without restricting micronutrients or any other nutrients such as vitamins. The reproducibility, consistency, and effectiveness of calorie restriction make it the most powerful and diversified laboratory maneuver in the field of experimental gerontology (2, 3). The well-recognized antiaging actions of calorie restriction are: (i) prevention of age-related functional deterioration, (ii) retardation of onset or progression of age-related diseases, and (iii) extension of both mean and maximum life span. Examples of these effects are listed in Table II. So far, slowed wound healing and low cold tolerance are the only known exceptions to the beneficial effects of calorie restriction.

Which Is More Effective, Restriction of Dietary Components or Total Calories?

Earlier nutritional experimentation produced data suggesting that restriction of specific dietary components, not calorie restriction per se, might also be responsible for modulating life span and other age-related changes. This suspicion arose because restricted food intake also reduced intake of other dietary components. Now several recent studies from our laboratory have produced interesting data showing effects of calorie restriction discernible from the effect of dietary components on longevity and disease process, and data are as follows.

Table II. Modulation of Age-Related Changes in Various Parameters by Calorie Restriction

A. Physiological and biochemical parameters
Serum cholesterol and triglyceride
Hormonal levels
Hormonal receptor activity
Protein biosynthesis and degradation
Blood glucose
Physical and locomotive activity
Collagen cross-linking
Immune competency
Reproductive activity
Bone metabolism
Prostaglandin metabolism
DNA synthesis and repair
Membrane structure and fluidity
Free radical and lipid peroxidation
Gene expression and transcription
Memory and neuronal function
Physical activity
B. Pathological disorders
Nephropathy
Cardiomyopathy
Leukemia
Tumorigenesis
Cataract
Amyloidosis

Effect of Fat and Minerals. We began a series of experiments in which the fat and mineral components of the diet were restricted by 40% (from 10% to 6% and from 5% to 3%, respectively) without restricting calories (17). The results on longevity indicate that neither the fat nor the mineral restriction modulated the median or maximum life spans of rats (Fig. 2). However, the pathological analyses revealed that fat, but not mineral, restriction did show some ability to retard the progression of nephropathy. It was concluded from this study that the high incidence of chronic nephropathy in aging male Fischer 344 rats can be improved by fat restriction without influencing life span, and that the 5% mineral consumption of *ad libitum* fed rats may not be the major factor contributing to age-related nephropathy seen in this strain of rats.

Effect of Soy Protein. Another important aspect of the study on dietary components is the question of whether the quality of dietary components (such as animal protein versus plant protein) has any impact on the progression of age-related disease and life span. We examined this question by investigating the effects of milk protein (casein versus soy protein) without changing caloric intake (23). Replacing dietary casein with soy protein improved median life span about 15% (Fig. 3). The pathological analyses indicate that this extended life span of 15% may be related to suppression of the progression of chronic nephropathy (Group 5 in Table III). In this case, only 7% of the rats on soy protein showed end-stage lesion of nephropathy, compared with about 40% of the rats on the casein diet. This beneficial effect on nephropathy was the major reason for the extended life span (Fig. 3). In addition, soy protein displayed other modulatory effects—for example, age-related hyperparathyroidism and senile bone loss (24). In the same study, the age-related elevation of serum parathyroid hormone was successfully prevented by soy protein diet (Fig. 4). A further benefit was an age-related decrease in serum 25-hydroxy vitamin D which was attenuated in soy protein fed rats (data not shown).

Further evidence was sought to establish which factor, calorie restriction or dietary component, is the dominating factor underlying these beneficial dietary effects (25). To obtain unequivocal proof, our laboratory initiated two different feeding experiments (26). One study involved the restriction of protein from 21% to approximately 12% without calorie reduction (Group 2 in Table III), which resulted in a 15% increase in life span of these rats. This experimental data revealed that the long-term intake of a diet containing 21% protein has a life span–shortening effect (as compared with Group 1 in Table III). Although a 15% life span extension is significant, it is not nearly as robust as a 50% increase by calorie restriction (Group 4 in Table III). In addition, pathological analysis revealed that the 15% increase in life span is largely due to the remedial effect of low protein intake on nephropathy (26). The level of 12% protein restriction did not influence any age-related physiological deterioration (26). In the second experimental study, further delineation of the efficacy of calorie restriction was sought by feeding calorie-restricted rats a diet containing 35% protein (Group 3 in Table III), an amount far exceeding that which should cause deleterious effects. Surprisingly, the life span of these rats (Group 3 in Table III) was the same as the calorie-restricted rats with 21% protein intake (Group 4, Table III), indicating no adverse effect of high protein intake. The protein intake of the rats in Group 3 was about 1.7 times higher than that of Group 4. The bottom line of these two studies is that calorie restriction is the overriding factor for modulating aging, age-related disease processes, and life span extension.

What Are Possible Mechanisms Underlying Calorie Restriction?

Intensive efforts of research on dietary restriction in the last several years have produced plausible hypotheses for the mechanisms. One of the most interesting of these is related to the free radical reaction. This hypothesis is based on the modern free radical theory of aging which provides a better mechanistic explanation of both the aging and age-related pathogenesis (see review Ref. 27). The free radical theory also provides the most reasonable cellular basis linking nutritional intervention of the aging process by dietary restriction with its antioxidant action.

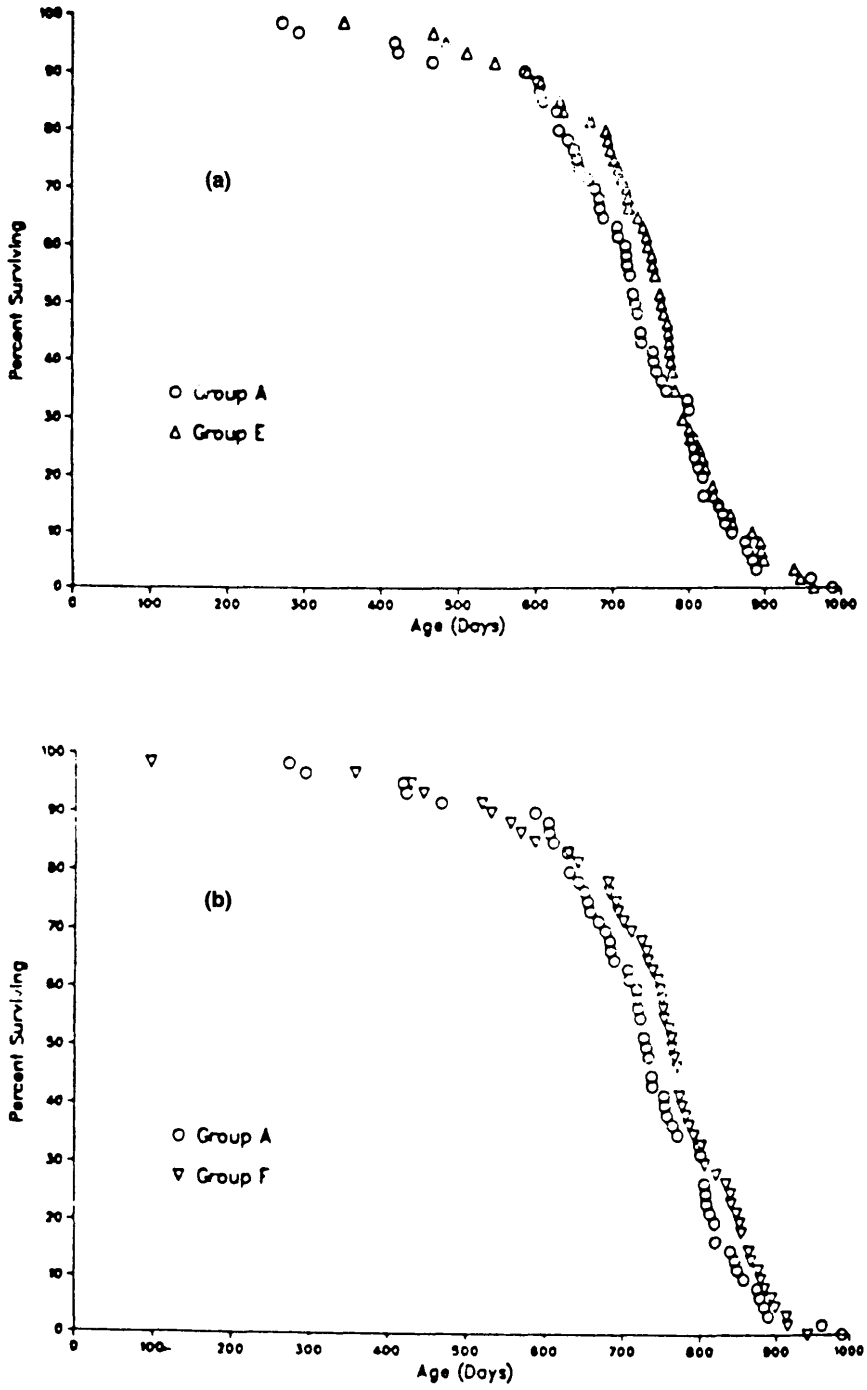

Figure 2. (a) Longevity curves for rats fed *ad libitum* (Group A) and rats fed a diet restricting fat intake by 40% (Group E). Reproduced with permission (25); (b) longevity curves for rats fed *ad libitum* (Group A) and rats fed a diet restricting mineral intake by 40% (Group F) (25).

Free Radical Involvement in Aging and Calorie Restriction

To have a better understanding of free radicals and their possible involvement in the aging process, it is worth describing some of the major aspects of free radical metabolism with respect to aging (28, 29). The aging processes and free radical reactions share common broad characteristics: (i) ubiquity. Aging seems to be a universal occurrence in all living things that depend on oxygen. Free radical reactions are inevitable consequences of oxygen metabolism and biological redox systems; (ii) progressiveness. Both processes are time-dependent, self-propagating, and continuing biological events; (iii) deleteriousness. The consequences of biological changes due to aging and free radical re-

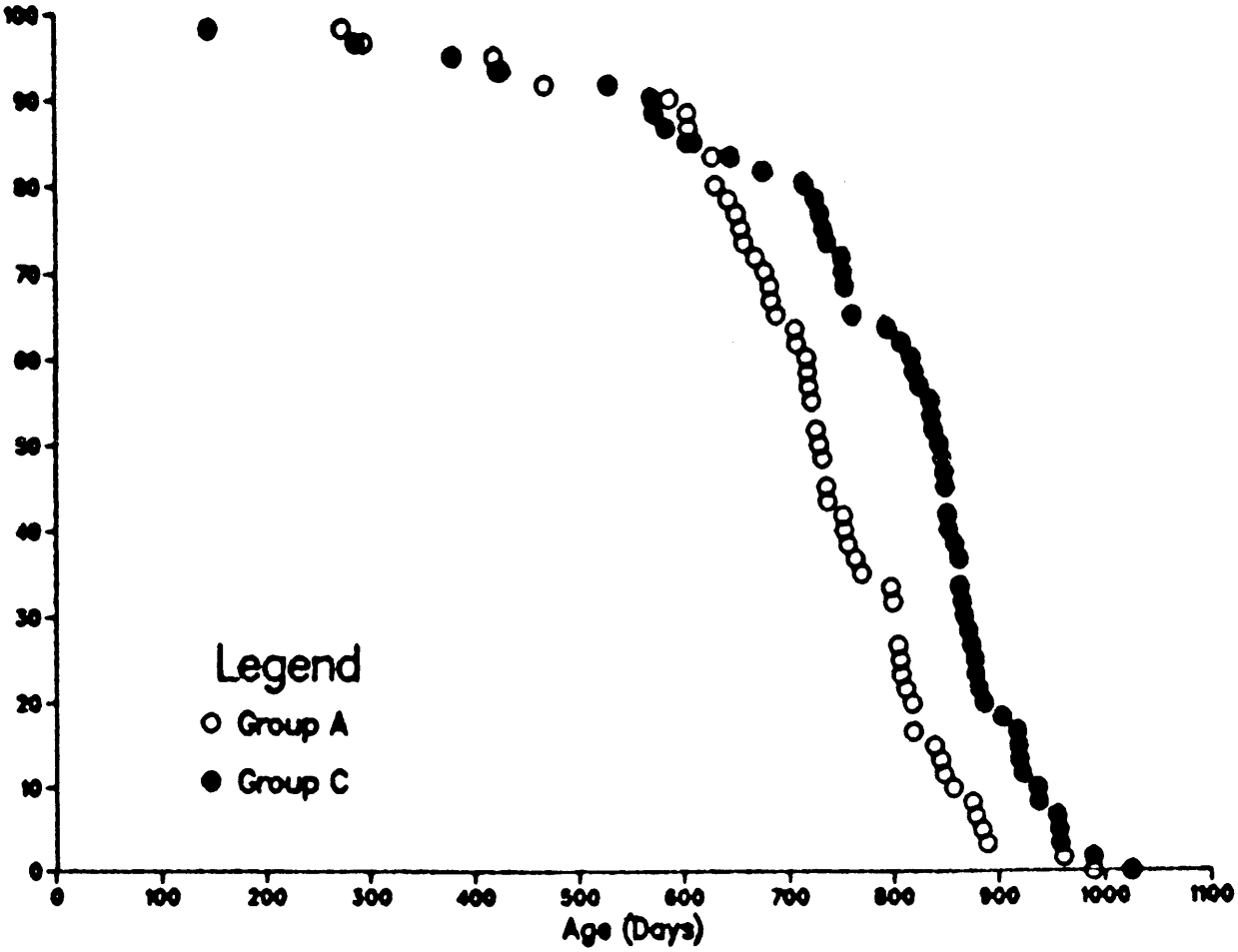

Figure 3. Longevity curves for rats fed *ad libitum* (Group A) and rats fed a diet containing 21% soy protein (Group C). Reproduced with permission (23).

Table III. Dietary Effect on Nephropathy and Life Span

Group	Diets	Nephropathy	Life span extension (over control)
1 (Control)	*Ad lib* feeding with casein (21%)	Severe	—
2	*Ad lib* feeding with casein restriction (12.5%)	Mild	15%
3	Calorie restriction with casein (35%)	Minimum	50%
4	Calorie restriction with casein (21%)	Minimum	50%
5	*Ad lib* feeding with soy protein (21%)	Mild	15%

actions are shown to be cumulative, thus leading to further functional loss and pathogenesis.

As we know, in all chemical reactions, free radicals are the most reactive atom or molecule species known to date existing in the biological systems (Table IV). Counteracting the problem of free radical reactivity is the extent of free radical protection. It is also well documented that under normal physiological conditions, an estimated 1%–3% of respired oxygen is converted to superoxide radicals ($O_2^{\cdot -}$) by mitochondria. This ubiquitous, inherent free radical threat is one of the strongest bases supporting the free radical theory of aging. Table V shows some of the free radical species known to play major roles in biological damage and perhaps in the aging process.

The extremely reactive nature of all the free radicals as defined depends on the unpaired electron, as evidenced by an unusually short half-life (Table IV). This unique chemical instability allows free radicals to induce oxidative alterations of biological molecules, leading to destruction or deterioration of function. Propagation of free radical reactions is further accelerated not only by reactive radicals but also by such catalysis as transition metals, Fe and Cu (31). It is known that hydrogen peroxide can be converted by hydroxyl species by Fe^{2+} or similar lipid hydroperox-

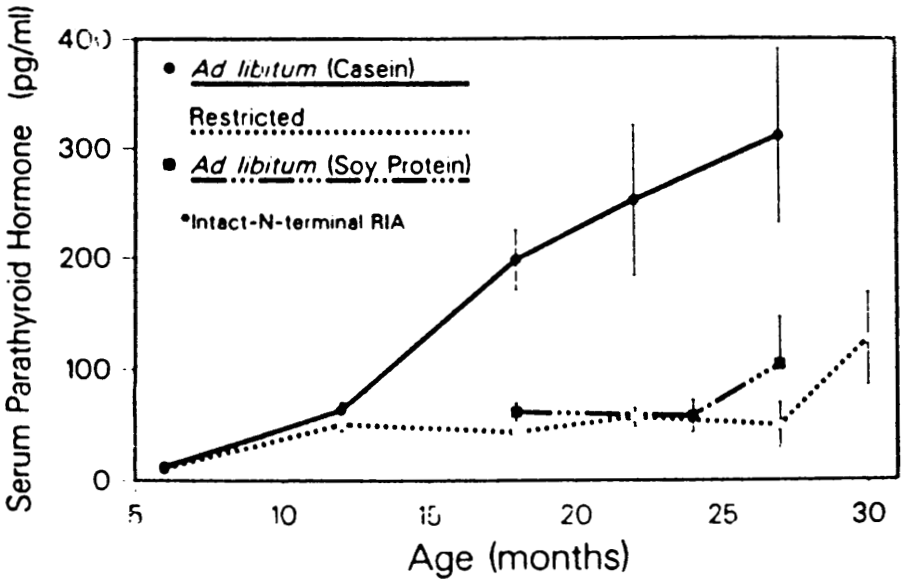

Figure 4. Effect of aging and dietary manipulation on serum parathyroid hormone. Reproduced with permission (24).

Table IV. Lifetimes of Free Radicals[a]

Species	Symbol	Half-life (sec) at 37°C
Superoxide	$O_2^{\cdot -}$	1×10^{-6}
Hydroxyl	$^{\cdot}OH$	1×10^{-9}
Alkoxyl	$RO^{\cdot}$	1×10^{-6}
Peroxyl	$ROO^{\cdot}$	1×10^{-2}
Singlet oxygen[b]	$^1O_2$	1×10^{-6}

[a] Adapted from Florence TM. Proc Nutr Aust **15**:88–73, 1990.
[b] Singlet oxygen is included to serve as comparison.

ides to peroxy radicals. The involvement of transition trace metals in free radical reactions is considered additional evidence linking nutritional influences on aging and pathogenic conditions such as Parkinson's disease (32, 33).

Cytosolic Defense Systems

Protection against such destructive processes is possible only by biologically active defense systems against oxidative stress, and all cells are endowed with well-balanced antioxidant defense systems. Existence of antioxidant defenses against oxygen and other radical species were evolutionarily developed to combat the incessant threat as a means of survival. According to the free radical theory, then, the optimum defense systems are essential biological components to sustain normal life and to attenuate free radical damage during aging. Among the important and well-known components of the defense systems are superoxide dismutase, catalase, and peroxidases. Unfortunately,they are shown to be deteriorated during age (34, 35).

Recent findings on the effects of calorie restriction on free radical reactions and antioxidant defense systems are consistent with the notion that modulation of free radicals is the mechanism by which calorie restriction exerts its antiaging action (34, 35). Specifically, calorie restriction shows (i) reduced free radical production (35), (ii) suppressed free radical–related damage (37–40), and (iii) prevention of age-related deterioration of cytosolic defense systems (34,35) as summarized in Table VI. Data are also available that show lipid peroxidation increased and selected cytosolic scavenging enzymes decreased with age (35, 41). Table VI lists several major free radical scavenging enzymes shown to be deteriorated in the aged cell, but remarkably these are well maintained by calorie restriction (42). Although the precise mechanisms are unknown, the antioxidant effects of calorie restriction may be related to the cell's ability to efficiently replace oxidatively damaged cellular constituents and altered enzyme molecules as suggested by several investigations (43–46). A review article covering many aspects of the effect of calorie restriction on the modulation of free radical metabolism was recently published (42).

Evidence of antioxidative action by calorie restriction was further strengthened by recent findings (40, 48, 49) on the protection of genomic components from age-related damage by oxidative alteration (Table VI). This genoprotection could be very significant in view of the fact that DNA damage may lead to serious consequences in pathogenesis of many chronic diseases, and in the enzyme biosynthesis and gene regulation such as those involved in cytosolic defense enzymes (43).

Modulation of Free Radicals and Age-Related Diseases by Calorie Restriction

Incidence of degenerative diseases such as atherosclerosis, diabetes, and cancer increases exponentially

Table V. Characteristics of Reactive Oxygen Species[a]

Species	Symbol	Properties
Superoxide anion	$O_2^{\cdot -}$	Good reductant, poor oxidant.
Hydroxyl radical	$HO^{\cdot}$	Extremely reactive (addition, abstraction and electron transfer reactions). Very low diffusion distance.
Perhydroxyl radical	HO_2	Stronger oxidant and more lipid soluble than superoxide. May initiate lipid peroxidation.
Peroxyl radical	$ROO^{\cdot}$	Low oxidizing ability relative to $HO^{\cdot}$ but great diffusibility.
Alkoxy radical	$RO^{\cdot}$	Intermediate in their reactivity with lipid between $ROO^{\cdot}$ and $HO^{\cdot}$.
Hydrogen peroxide	H_2O_2	Oxidant but reactions with organic substrates are sluggish. High diffusion capability.
Singlet oxygen	$^1O_2$	Powerful oxidizing agent with halftime of 10^{-6} sec.

[a] Adapted from Ref. 30.

Table VI. The Effect of Age and Dietary Restriction on Age-Related Changes in Free Radical Metabolism

	Age	Dietary restriction
Free radical and H_2O_2 production[a]		
Superoxide	No change	Decrease
Hydroxyl	No change	Decrease
H_2O_2	No change	Decrease
Cytosolic antioxidant defense[b]		
Superoxide dismutase[a]	No change	Increase
Catalase	Decrease	Increase
Reduced glutathione	Decrease	Increase
GSH reductase	Decrease	Increase
GSH peroxidase	Decrease	Increase
MDA elimination by mitochondrial oxidation[c]	Decrease	Increase
Genomic and protein alterations		
DNA damage[d]	Increase	Decrease
DNA repair[e]	Decrease	Increase
Protein oxidation[f]	Increase	Decrease

[a] Liver microsomes (35).
[b] Liver cytosols (41).
[c] Liver mitochondria (36).
[d] Liver (40).
[e] Hepatocyte (49).
[f] Liver tissue (45).

Table VII. The Effect of Age and Dietary Restriction on Age-Related Changes in Membrane Structure

Membrane component	Change with age	Dietary restriction
Membrane component		
Lipids[a]		
Hydroperoxide	Increase	Decrease
Fatty acids	Increase	Decrease
18:2	Decrease	Increase
20:4; 22:5; 22:6	Increase	Decrease
Peroxidizability index	Increase	Decrease
MDA production	Increase	Decrease
Protein[b]		
Cytochrome P-450	Decrease	Increase
Cytochrome b_5	No change	Increase
Membrane property		
Membrane fluidity[b,c]	Decrease	Increase
Membrane transition temperature[b]	Increase	No change

[a] Liver and kidney mitochondria and microsomes (38).
[b] Liver mitochondria and microsomes (60).
[c] Liver microsomes (61).

with age. In fact, aging has been considered to be the most important risk factor in all three diseases in humans. The etiologies of these diseases are known to be multiple and diverse, but recent data provide cogent evidence for intimate involvement of free radicals as causal factors or major participants in the disease process. There are already several proposals in the literature on the pathogenesis of these diseases based on free radical hypotheses—for instance, the Oxidative Modification Hypothesis for atherosclerosis (50), Glycation Hypothesis for diabetes (51), and Initiation and Promotion Hypothesis for neoplastic disease (52, 53). Possible common processes linking free radical reactions to these diseases have been recently proposed (54).

Dietary modulation of these chronic age-related pathogeneses may be closely related to the fact that dietary restriction exerts an antioxidant action against lipoperoxidation, free radical-mediated glycation, and DNA damage, which are known to play a major role in increasing incidence of these life-shortening diseases. For instance, it has been shown that calorie restriction markedly reduces serum cholesterol and triglyceride (55), and lipid peroxidation (34, 38). Calorie restriction as a possible antidiabetogenic modulator has already been shown to reduce levels of plasma, glucose, and glycated hemoglobin (56), thereby attenuating the extent of glycation and free radical–mediated glucose autoxidation. Similarly, the antitumor action of calorie restriction exemplified by a lower incidence of tumorigenesis in the aged rodents has been well established (23, 25). A recent report of Chou *et al.* (57) reinforces the possibility that *in vivo* challenge with a potent carcinogen agent, aflatoxin B1, causing DNA strand breaking was significantly prevented by dietary restriction, showing reduced nuclear DNA binding of the toxin.

Another example of a disease in which free radi-

cals are likely to be causal factors is Parkinson's disease. The Oxidant Stress Hypothesis of Parkinson's disease, a proposal of Fahn (58), strongly indicates the deleterious effect of free radicals through dopamine oxidation on the disease process. In addition, the generation of H_2O_2 by oxidation of dopamine by monoamine oxidase will cause increased oxidative neuronal damage by iron-catalyzed hydroxyl radical formation from H_2O_2. In further support of this possibility are two new pieces of evidence: the age-related accumulation of iron in striatal neurons (32) and the presence of neuromelanins which are known to regulate iron homeostasis (33). This free radical-based hypothesis is the most appealing molecular explanation as to why and how nigral neurons are more vulnerable to the age-related insult (59). Further substantiations by recent experimental studies are beginning to delineate the precise mechanisms and the extent of free radical involvement in many chronic diseases. New evidence generated from recent studies is persuasive, perhaps making the free radical hypothesis the best model available at present. Given the antioxidant action of calorie restriction and its effectiveness in the maintenance of cellular homeostasis in the rodent model, it is quite understandable how chronic degenerative diseases could be ameliorated by proper nutritional intervention. One big question remaining for investigators to answer is how calorie restriction reduces overall oxidative stress and enhances antioxidant defense systems during senescence. This question will be one of the major future research directions of free radical biology in gerontology.

Dietary restriction has served as a tool to explore the interaction of nutrition, aging, and pathogenesis. It seems clear that nutrition can modulate life span by influencing the biological process, pathological process, or both. The quantitative measurement of the extent of such interactions is difficult to ascertain at present, but a hypothesis has been forwarded to propose modulation of free radical metabolism by calorie restriction as the underlying mechanism.

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