Dietary Restriction Downregulates Free Radical and Lipid Peroxide Production: Plausible Mechanism for Elongation of Life Span

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Summary Dietary restriction elongates life span by suppressing age-related diseases in experimental animals. It has received a great deal of attention in connection with the relationship between aging, nutrition, and oxidative stress because oxidative injury in several tissues is a prominent feature in the aging process. Although the oxidative stress theory of aging has currently gained popularity, the premise from which this hypothesis was derived is paradoxical because the same oxygen, that supports life in one hand threatens survival and promotes aging in the other. Until recently, no single experimental paradigm could offer satisfactory mechanistic explanations for this complex issue. Recent investigations using the life-extending dietary restriction regimen could offer satisfactory mechanistic explanations for this apparent self-contradiction to life. The modulation of free radical-induced oxidative stress provided sufficient data to support the notion that dietary restriction’s antiaging effect may come from its ability to tightly regulate the oxidative status of an organism. The result is the maintenance of cellular homeostasis, a hallmark of dietary restriction’s action in the extension of life span. To date, we reported that dietary restriction (maintained on 60% of ad libitum feeding) suppresses age-related oxidative damage by modulating the amount as well as the fatty acid composition of tissue phospholipids. These remarkable findings have been incorporated into the new “membrane peroxidation cycle” concept. The intervention of this cycle appears to be an evolutionary process that the dietary restricted rats have adapted as a strategy to protect the membrane in an oxidative environment.

Key Words aging, dietary restriction, fatty acid composition, oxidative stress, membrane peroxidation cycle

Aging can be defined as the accumulation of deleterious changes that increase the risk of death. These changes can be attributed to both the genetic and the various environmental factors (1) and will inevitably compromise an organism’s ability to meet both internal and external challenges. Oxidative stress, a prime example of such alterations, is causally related to irreversible damages resulting from endogenously generated free radicals (2, 3). The condition becomes worsened by an age-related decline in the organism’s defense against these challenges (4, 5). These phenomena result in redox imbalance that is referred as oxidative stress.

A reduction of the caloric intake of laboratory animals, while maintaining nutrition, can increase their life spans (6–8). Dietary restriction delays age-associated pathological and physiological changes (7–9). One major mechanism by which dietary restriction retards the aging process is its remarkable ability to reduce oxidative damages (5–10). For example, Kim et al. (11) have shown that dietary restriction decreases the malondialdehyde content of cardiac mitochondria, indicating a decrease in lipid peroxidation. Restricted feeding regimens enhance the organism’s ability to attenuate levels of harmful reactive-free radicals in various organs (12, 13). Oxidative stress-mediated injury is causally related to aging, since reactive oxygen species (ROS)-caused damages accumulate with age (14). Although several previous studies have reported a relationship between aging and lipid peroxidation (15), most have examined TBARS levels to indicate peroxidative status. Furthermore, we determined phospholipid hydroperoxide (PLOOH) as a sensitive, key indicator for oxidative injury by using a chemiluminescence (CL)-HPLC, because phospholipids are important structural and func-
tional components of the biological system (16, 17) and are commonly recognized as the major target of lipid peroxidation.

To assess age-related phospholipid peroxidation, we used the flow injection-chemiluminescence (FI-CL) method that has been developed to monitor active oxygen-derived radical activity (18). Previous reports from our laboratory described a simple and continuous one-step flow injection system based on cytochrome c-amplified chemiluminescence for the assay of radical scavenging activity (18, 19). The present review deals with an attempt to quantify age-associated changes of the lipid profile and the degree of attenuation of oxidative damage by dietary restriction.

The Effect of Dietary Restriction on Active Oxygen-Derived Radical Levels and Membrane Fatty Acid Composition

We demonstrated that hepatic oxidative stress increases with age in ad libitum fed rats, but less in dietary restricted rats. The index of linoleic acid desaturation and the unsaturation degree of fatty acids in hepatic PC and PE were significantly reduced by dietary restriction (20). Several investigators have examined the effect of dietary restriction with age on lipid peroxide concentrations, mostly by using TBARS (14, 21). These studies suggest that fatty acid unsaturation is a main factor in determining the sensitivity to lipid peroxidation. To evaluate the inhibitory effect of dietary restriction on oxidative stress, we measured PCOOH as a sensitive marker because PC is vulnerable to peroxidation (22). Male weaning rats were fed commercial chow for 4 and 12 months and were subjected to lipid analyses.

The concentration of phospholipid hydroperoxide, prominently PCOOH, increased significantly with age in ad-libitum-fed rats (Fig. 1). This increase was significantly reduced by dietary restriction. The same trend was confirmed in the TBARS values. Thus dietary restriction appears to effectively suppress free radical production at the tissue levels as age increased. These results show that dietary restriction significantly reduces lipid damages.

However, no effect of feeding manipulation was observed on catalase and superoxide dismutase (SOD) activities (Fig. 2). The activities of these enzymes apparently did not change with age. It is interesting that significant changes occurred in liver cholesterol and triglyceride levels as a result of dietary restriction.

Fig. 1. Effects of dietary restriction on the production of phosphatidylcholine hydroperoxide (PCOOH; upper panel) and thiobarbituric acid-reactive substances (TBARS; bottom panel) in the liver of rats aged 4 (young) and 12 months (adult). Each bar represents the mean±SE of five rats. **p<0.01, ***p<0.001 compared with young and adult groups. +p<0.05, ++p<0.01, +++p<0.001 compared with ad libitum (AL) and dietary restriction groups (from Jeon Tae Il, Lim BO, Yu BP, Lim YH, Jeon EJ, Park DK. 2001. Lipids 36: 589–593).

Fig. 2. Effects of diet restriction on the cytosolic catalase activity (upper panel) and SOD activity (bottom panel) in the liver of rats aged 4 (young) and 12 months (adult). Each bar represents the mean±SE of five rats. No significant difference was found between young and adult groups. No significant difference was found between ad libitum (AL) and dietary restriction groups (from Jeon Tae Il, Lim BO, Yu BP, Lim YH, Jeon EJ, Park DK. 2001. Lipids 36: 589–593).
Dietary restriction suppressed the age-related increase in these lipids, but not phospholipid (Fig. 3). The concentration of phospholipid tended to increase after dietary restriction. To further explore the notion that tissue lipid vulnerability is a crucial factor contributing to cellular oxidative status, constituent fatty acids in PC and PE were analyzed. The trends in fatty acid composition to increase toward peroxidizable polyunsaturated fatty acid (PUFA) with age are evident (Table 1). The proportion of PUFA such as 20:4n-6 and 22:5n-6 in liver PC tended to be low in dietary restricted rats at 4 and 12 months. This was not so for liver PE in which 22:6n-3 decreased by dietary restriction. In contrast, 18:2n-6 increased after restricted feeding in both ages of rats in both phospholipids. Thus major PUFA were demonstrably lowered by dietary restriction in adult rats. Among the most obvious modifications by dietary restriction has been the compositional changes related to membrane lipid composition, specifically the age-related membrane fatty acid composition (23). It has been proposed that the dietary restriction modulation of the fatty acid profile is to resist age-related oxidative stress and insults as a possible adaptive strategy (10, 12, 15). In our study, 18:2n-6 and 18:3n-3 increased in dietary restricted rats, but the content of PUFA derivatives (20:4n-6, 22:5n-6 and 22:6n-3) decreased (Table 1). The effects on the changes in unsaturated fatty acid regarding oxidative damage are more noticeable when the number of double bonds is more than 4 per fatty acid chain, though the response pattern differed to some extent between PC and PE.

The membrane fatty acid composition affects the degree of peroxidizability: The higher the unsaturated the fatty acid, the greater its peroxidizability (10, 15, 24–26). The significance of this effect has become more evident, and increased PUFA levels with age are causally related to age-related oxidative lipid damage (15). The results demonstrate that dietary restriction modulates PUFA content by reducing arachidonic acid (20:4n-6) in liver PC and docosahexaenoic acid (22:6n-3) in liver PE. This pattern of modulation is expected to increase the resistance to oxidative stress by attenuating lipid peroxidation (24, 27). Dietary restriction is thus very likely to exert its antiaging action on membrane stability by maintaining the integrity of PUFA (27).

We suggest that dietary restriction leads to a protection of organ in vivo against age-related increases of oxidative stress by modifying lipids and their composition to reduce peroxidizable substrates such as PUFA in phospholipid. Also, the results are consistent with earlier data (7, 12, 15, 24) that dietary restriction is a potential antioxidative strategy to alleviate liver oxidative injury, thereby slowing the progression of aging.

### The Interference with Dietary Restriction of the Membrane Peroxidation Cycle

The diverse effects of dietary restriction on the modulation of free radical-related processes are summarized in Table 2. As an outstanding membrane modifier, the ability of dietary restriction to ward off age-related membrane deterioration is the most suitable mechanistic explanation for the oxygen paradox. Among the most obvious modifications by dietary restriction are compositional changes related to membrane lipid composition, specifically, age-related change in the membrane fatty acid composition (10, 28). This is an intriguing phenomenon because until recently it was unheard for reduced calorie intake to alter membrane composition, though various dietary sources are known to change membrane compositional changes, repelling oxidative insults by suppressing the rise of polyunsaturation, which is compensated by increased 18:2 fatty acid. Evidence shows that during aging, the membrane fatty acid profile tends to increase toward peroxidizable polyunsaturated acids, such as 22:4n-6.
Table 1. Effect of dietary restriction (DR) on the fatty acid composition of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in the liver between ages 4 and 12 months of rats.

| Fatty Acids (weight %) | 4 mon | 12 mon | 4 mon | 12 mon |
|------------------------|-------|--------|-------|--------|
|            | AL    | DR    | AL    | DR    |
| C14:0      | 0.2±0.0a | 0.2±0.0a | 0.1±0.0a | 0.1±0.0a |
| C16:0      | 15.4±0.4b | 16.3±0.9b | 13.2±0.4b | 13.2±1.1b |
| C16:1      | 0.9±0.0c | 1.1±0.1c | 0.5±0.0b | 0.3±0.0a |
| C18:0      | 25.1±0.8c | 24.4±1.5c | 27.6±5.1b | 24.2±2.1b |
| C18:1n-9   | 2.7±0.1c | 3.6±0.3c | 2.2±0.1a | 2.6±0.2b |
| C18:2n-6   | 11.3±0.9c | 15.2±0.7c | 8.8±0.3b | 11.1±0.9b |
| C18:3n-3   | 0.3±0.0c | 0.4±0.0c | 0.2±0.0a | 0.3±0.0c |
| C20:3n-6   | 0.7±0.1c | 1.2±0.1b | 0.5±0.0a | 0.4±0.1a |
| C20:4n-6   | 31.8±0.6c | 28.1±1.0c | 34.0±0.3c | 30.7±2.7b |
| C22:4n-6   | 0.9±0.0b | 0.8±0.1a | 1.0±0.1b | 1.0±0.1b |
| C22:5n-6   | 2.3±0.3b | 1.4±0.1c | 4.3±0.3c | 2.3±0.4b |
| C22:5n-3   | 0.6±0.0b | 0.7±0.1b | 0.4±0.0a | 0.6±0.1b |
| C22:6n-3   | 3.0±0.1c | 2.9±0.3a | 3.3±0.1a | 2.6±0.3c |
| (C20:3+20:4)| 3.0±0.3c | 1.9±0.1a | 3.9±0.1d | 2.8±0.1b |

Values represent the mean±SE of five rats. Values in a row with a different superscript are significantly different (p<0.01).

AL, ad libitum; DR, dietary restriction. (from Jeon Tae Il, Lim BO, Yu BP, Lim YH, Jeon EJ, Park DK. 2001. Lipids 36: 589–593).

Table 2. Factors influencing the modulation of oxidative stress by dietary restriction.

- A. Generation of reactive species
  - Superoxide
  - H2O2
  - MDa
  - 4-hydroxy nonenal
- B. Lipid peroxidation
- C. Defense systems
  - Superoxide dismutase
  - Catalase
  - Glutathione
- D. Iron accumulation
- E. Age-related membrane rigidity

and 22:5n-3 (10, 29). Changes in this profile can be taken as a compensatory mechanism for age-related membrane rigidity. However, the consequence of this compensatory increase in highly PUFAs is an even higher state of peroxidizability, leaving the membrane even more susceptible to oxidative attack with exacerbated membrane rigidity (28). This paradoxical and vicious cycle is termed the “membrane peroxidation cycle” (MPC, Fig. 4). Dietary restriction seems to modulate the fatty acid profile as a possible adaptive strategy to tolerate age-related oxidative damage, thereby preventing membrane deterioration (15).

An interesting action by dietary restriction is shown in its ability to intervene in the MPC by producing less highly peroxidizable acids, with an accompanying increase of linoleic acid at the site of the cycle. In other words, the significance of an increase in 18:2n-6 is that it is seemingly an adaptive action by the organism to properly maintain membrane fluidity without the risk of increased peroxidizability—an efficient, smart strategy to resolve the oxidative paradox. This strategy seems to develop in an evolutionary manner as an adaptive measure to fight oxidative damages, as shown in works by Barja’s group (15). These investigators have shown differences in the life span of rats, pigeons, and humans through comparison studies of the degree of unsaturated fatty acid contents among these species. Their findings show that the extent of polyunsaturation (i.e., peroxidizability) inversely correlates with longevity (i.e., the higher the unsaturation, the shorter the life span), as was seen in short-lived rats compared with humans. Leading the way to the resolution of another paradox of aging, the species comparison shows that pigeons live eight to nine times longer than rats, despite having higher metabolic rates, which leads to the conclusion that a pigeon’s longevity may be primarily attributed to increased resistance to lipid peroxidation as a result of lower polyunsaturated fatty acid levels. The dietary restriction paradigm seems to mimic an adaptive, evolutionary strategy for the survival of the organism when it is faced to the oxidative stress.

Because membrane integrity is an essential requirement of cellular homeostasis, it seems that maintaining it should be an organism’s priority for survival, even during limited calorie availability, as with dietary restriction. This survival strategy by an organism falls well within Kirkwood’s general idea of the disposable soma theory, which proposes that an organism prioritizes energy utilization to maintain longevity of the species, which is necessary for survival (30). Certainly,
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Fig. 4. Proposed scheme of the membrane peroxidation cycle. The increased lipid peroxidation resulting from age-related compositional changes in polyunsaturated fatty acids causes membrane rigidity. To maintain and compensate membrane fluidity, more polyunsaturated fatty acids are synthesized, causing the membrane to become more vulnerable to peroxidation. Dietary restriction intervenes in this vicious cycle by maintaining membrane lipid composition with less peroxidizable fatty acids.

Fig. 5. Comparison of the effects of peroxidized and cholesterol-loaded liposomes on the membrane fluidity of microsome (from AL fed rats at 6 months); \( \blacktriangle \) oxidized-liposome; \( \blacktriangle \) cholesterol-loaded liposome; letters a and b denote significant differences (\( p<0.05 \)) (from Choe M, Jackson C, Yu BP. 1995. Free Radic Biol Med 18: 977-984).

without properly maintained essential cellular membrane integrity, survival is not possible (5, 28, 31).

Observations made in our laboratory during the past few years show substantial age effects in the mitochondrial and microsomal membranes. As shown in Fig. 5, when the effect of peroxidation on membrane fluidity was compared with cholesterol loading, membrane fluidity was amazingly sensitive to peroxidation, with markedly increased rigidity as a result of the incorporation of peroxidized phospholipids. This exquisite sensitivity to the structural changes of the membrane lipids was contrasted with resistive changes toward the compositional alterations by cholesterol incorporation. In our experiment, even at the highest C/P ratio of .445, which is very unlikely to occur under normal physiological conditions, the reduction in fluidity (to 3.17, from 1/P 3.27) represents only a 48% change induced by peroxidation. We found further evidence to support dietary restriction’s preventive action by documenting that age-related membrane rigidity is due to an extreme sensitivity to membrane lipid peroxidation. Contradicting the traditional view of age-related membrane rigidity, our studies show that the membranes of dietary-restricted rats remain fluid even when the cholesterol, triglyceride, and phospholipid increase with age (20, 24, 32).

Oxygen Paradox: Physical Exercise and Dietary Restriction

Improved sanitation, personal hygiene, the eradication of infectious disease, and the elimination of malnutrition have all contributed to increased life span. Yet modern conveniences have increased morbidity as populations have adopted sedentary lifestyles because of reduced physical activity. Our current sedentary lifestyles are considered a major underlying risk factor for disability in the elderly. There is little doubt that well-maintained physical activity is the best way to reduce the incidence of cardiovascular disease, diabetes, obesity, and other adult-onset disabilities. Rat experimentation clearly shows that exercise extends life span (33). Yet its metabolic demands require high oxygen consumption, which causes possible oxidative damage—another oxygen paradox. As we might expect, the production of reactive oxygen species in mitochondria in-
Fig. 6. Resistance to tert-butylhydroperoxide challenge in mitochondria isolated from dietary restriction rats. The indicated time for permeability transition was much prolonged compared to ad-libitum-fed controls. This shows the dietary restriction’s ability to maintain a more stabilized membrane structure through increased resistance as a result of an increased resistance to oxidative stress. Ad libitum fed controls; Dietary restricted rat. ** p<0.05, *** p<0.001 (from Yu BP, Lee DW, Hwang EH, Lim BO, 1999. In: The Paradoxes of Longevity (Robine JM, Forette B, Franceschi C, Allard M, eds), p 93–102, Springer-Verlag, Berlin, Heidelberg, New York).

Fig. 7. Suppression of microsomal production of reactive oxygen species by dietary restriction. AS, ad-libitum-fed, sedentary; AE, ad-libitum-fed, exercising; RS, dietary restricted, sedentary; RE, dietary restricted, exercising. DCF=2',7'-dichlorodihydrofluorescin. (from Yu BP, Lee DW, Hwang EH, Lim BO. 1999. In: The Paradoxes of Longevity (Robine JM, Forette B, Franceschi C, Allard M, eds), p 93–102. Springer-Verlag, Berlin, Heidelberg, New York).

Fig. 8. Enhanced membrane fluidity by exercise. AS, ad-libitum-fed, sedentary; AE, ad-libitum-fed, exercising; RS, dietary restricted, sedentary; RE, restricted exercising (from Yu BP. 1996. Free Radic Biol Med 21: 651–668).

Increases during exercise. This phenomenon of the oxygen paradox has been observed in increased lipid peroxidation (34) and in DNA damage (35) detected in exercising subjects. It is important to note, however, that, under proper conditions, this oxidative damage is shown to be sizably attenuated by the counteraction of the antioxidative defense systems, which are stimulated by exercised-induced oxidative stress. Thus the defense mechanism plays a key role in resolving the exercise oxygen paradox.

Coupled with physical exercise, the dietary restriction paradigm provides another interesting way to explain the apparent oxygen paradox. A recent study (36) reported data showing that dietary-restricted animals that exercise have an additionally extended mean life span compared to their nonexercising dietary-restricted counterparts. How could this be possible if exercise promotes oxidative damage? The answer lies in the dietary restriction’s unique ability to withstand oxidative challenge. As the data show (Fig. 6), the mitochondria permeability of dietary restricted rats was protected. Another interesting antioxidative action of dietary restriction is its ability to defend against the generation of damaging ROS, which is expected to increase with exercise but is actually suppressed by dietary restriction (Fig. 7). It is interesting that the synergistic effect of the combined intervention of dietary restriction and exercise is exemplified by the preservation of membrane fluidity (Fig. 8). When this is all put together, the evidence shows that the exercising dietary-restricted rats, despite having increased mitochondrial ROS production, have stronger mechanisms to defend against oxidative stress than the nonexercising dietary-restricted rats do. The mechanisms for this remarkable resistance to oxidative challenge is very likely to come from the concerted effort of enhanced antioxidative defense systems (5) and the suppression of peroxidizability of membrane fatty acids, as indicated in the intervention in the membrane peroxidation cycle by dietary restriction.
Conclusion

Theories and concepts of aging inundate the field of gerontology. Significant is the emergence of the free-radical-based oxidative theory of aging, which encompasses many existing theories. Theories relative to glycation, mutation, DNA damage, and nitric oxide could all fall under the oxidative stress theory. It is intriguing to know that this most versatile theory is at least fully supported by the modulation of membrane composition and the antioxidant defense essential to the maintenance of cellular homeostasis. In this review, "membrane peroxidation cycle," because of the effects brought about by dietary restriction, was discussed to highlight an adaptive membrane-stabilizing strategy for longevity, much like the evolutionary process observed in long-lived species. The dietary restriction paradigm deserves recognition not only for its protective effect in maintaining cellular homeostasis by attenuating oxidative stress, but also for its ability to modulate the "membrane peroxidation cycle," thereby providing us with a mechanistic explanation for some of the apparent paradoxes that exist in the aging processes.

REFERENCE

1) Harman D. 1981. The aging process. Proc Natl Acad Sci USA 78: 7124–7128.
2) Reiter RJ. 1995. Oxidative processes and antioxidative defense mechanisms in the aging brain. FASEB J 9: 526–533.
3) Yu BP. 1994. Cellular defenses against damage from reactive oxygen species. Phys Rev 74: 139–162.
4) Baker GT, Martin GR. 1994. Biological aging and longevity: Underlying mechanisms and potential intervention strategies. J Aging Phys Act 2: 304–328.
5) Yu BP. 1996. Aging and oxidative stress: modulation by dietary restriction. Free Radic Biol Med 21: 651–668.
6) Weindruch R, Walford RL, Fligiel S, Guthrie D. 1986. The retardation of aging in mice by dietary restriction: Longevity, cancer, immunity and lifetime energy intake. J Nutr 116: 641–654.
7) Lane MA, Ingram DK, Roth GS. 1999. Nutritional modulation of aging in nonhuman primates. J Nutr Health Aging 3: 69–76.
8) Roth GS, Ingram DK, Lane MA. 1999. Calorie restriction in primates: Will it work and how will we know? J Am Geriatr Soc 47: 896–903.
9) Choi YS, Goto S, Ikeda I, Sugano M. 1988. Age-related change in lipid metabolism in rats: the consequence of moderate food restriction. Biochim Biophys Acta 963: 237–242.
10) Laganierre S, Yu BP. 1993. Modulation of membrane phospholipid fatty acid composition by age and food restriction. Gerontology 37: 7–18.
11) Kim JD, Yu BP, McCarter RJM, Lee SY, Herlihy JT. 1996. Exercise and diet modulate cardiac lipid peroxidation and antioxidant defenses. Free Radic Biol Med 20: 83–88.
12) Kang CM, Kristal BS, Yu BP. 1998. Age-related mitochondrial DNA deletions: effect of dietary restriction. Free Radic Biol Med 24: 148–154.
13) Yu BP, Yang R. 1996. Critical evaluation of the free radical theory of aging: A proposal for the oxidative stress hypothesis. Ann NY Acad Sci 786: 1–11.
14) Baek BS, Kwon HJ, Lee KH, Yoo MA, Kim KW, Ikeno Y, Yu BP, Chung HY. 1999. Regional difference of ROS generation, lipid peroxidation, and antioxidant enzyme activity in rat brain and their dietary modulation. Arch Pharm Res 22: 361–366.
15) Pamplona R, Prat J, Cadena S, Rojas C, Perez-Camp R, Lopez M, Barja TG. 1966. Low fatty acid unsaturation protects against lipid peroxidation in liver mitochondria from long-lived species: The pigeon and human case. Mech Aging Dev 86: 53–66.
16) Miyazawa T, Suzuki T, Fujimoto K, Yasuda K. 1992. Chemiluminescent simultaneous determination of phosphatidylcholine hydroperoxide in the liver and brain of the rat. J Lipid Res 33: 1051–1059.
17) Terao J, Piskula M, Yao Q. 1994. Protective effect of epicatechin, epicatechin gallate and quercetin on lipid peroxidation in phospholipid bilayers. Arch Biochem Biophys 308: 278–284.
18) Choi HY, Jhun EJ, Lim BO, Chung JM, Kyung SH, Park, DK. 2000. Application of flow injection-chemiluminescence to the study of radical scavenging activity in plants. Phytother Res 14: 250–253.
19) Choi HY, Song JH, Park DK. 1998. A combined flow injection-chemiluminescence method for the measurement of radical scavenging activity. Anal Biochem 264: 291–293.
20) Jeon TI, Lim BO, Yu BP, Lim YH, Jeon EJ, Park DK. 2001. Effect of dietary restriction on age-related increase of liver susceptibility to peroxidation in rats. Lipids 36: 589–593.
21) Albrecht R, Pelissier MA, Atteba S, Smalli M. 1992. Dietary restriction decreases thio-barbituric acid-reactive substances generation in the small intestine and in the liver of young rats. Toxicol Lett 63: 91–96.
22) Miyazawa S, Suzuki T, Fujimoto K, Kaneda T. 1990. Phospholipid hydroperoxide accumulation in liver of rats intoxicated with carbon tetrachloride and its inhibition by dietary alpha-tocopherol. J Biochem 107: 689–693.
23) Pieri C. 1991. Food restriction shows down age-related changes in cell membranes. Ann NY Acad Sci 621: 353–362.
24) Choe M, Jackson C, Yu BP. 1995. Lipid peroxidation contributes to age-related membrane rigidity. Free Radic Biol Med 18: 977–984.
25) Song JH, Fujimoto K, Miyazawa T. 2000. Polyunsaturated (n-3) fatty acids susceptible to peroxidation are increased in plasma and tissue lipids of rats fed docosahexaenoic acid-containing oils. J Nutr 130: 3028–3033.
26) Choi JH, Yu BP. 1989. The effect of food restriction on kidney membrane structures of aging rats. Age 12: 133–136.
27) Lee JW, Yu BP, Herlihy JT. 1999. Modulation of cardiac mitochondrial membrane fluidity by age and calorie intake. Free Radic Biol Med 26: 260–265.
28) Pieri C. 1997. Membrane properties and lipid peroxidation in food restricted animals. Age 20: 71–79.
29) Devasagayam TPA. 1986. Low level of lipid peroxidation in newborn rats: Possible factors for resistance in hepatic microsomes. FEBS Lett 199: 203–307.
30) Kirkwood TBL. 1997. Is there a biological limit to the human life span? In: Longevity: To the Limits and Beyond (Robine LM, Vaupel JW, Jeune B, Allard M, eds), p 69–76. Springer-Verlag, Berlin.
31) Zs-Nagy I. 1994. The Membrane Hypothesis of Aging. CRC Press, Boca Raton, FL.

32) Yu BP, Suescun EA, Yang SY. 1992. Effect of age-related lipid peroxidation on membrane fluidity and phospholipase A2: Modulation by dietary restriction. *Free Radic Biol Med* **21**: 651–668.

33) Hollosy JO, Smith EK, Vining M, Adam S. 1985. Effect of voluntary exercise on longevity of rats. *J Appl Physiol* **59**: 826–831.

34) Ji LL, Leichtweis S. 1997. Exercise and oxidative stress: Source of free radical and their impact on antioxidant systems. *Age* **20**: 91–106.

35) Hartmann A, Nieb AM, Grunert-Fuch M, Poch B, Speit G. 1995. Vitamin E prevents exercise-induced DNA damage. *Mutat Res* **346**: 195–202.

36) McCarter RJM, Shimokawa I, Ikeno Y, Higami Y, Hubbard GB, Yu BP, McMahan CA. 1997. Physical activity as a factor in the action of dietary restriction on aging: Effects in Fischer 344 rats. *Aging Clin Exp Res* **9**: 73–79.