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Original Contribution

BRAIN SYNAPTOSOMAL AGING: FREE RADICALS AND MEMBRANE FLUIDITY

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Abstract—Dietary restriction was used in this study as a modulator of free radical reactions to examine the effects of age on the physico-biochemical property of synaptosomal membranes. Synaptosomal membranes were isolated from the frontal cortices of 6- and 24-month-old barrier-reared male Fischer 344 rats maintained on either an ad lib (AL) or a 40% diet restricted (DR) feeding schedule. The age-related production of reactive oxygen species (ROS) was only seen in the AL group, and dietary restriction suppressed the amount of the reactive species at both ages. Although membrane fluidity significantly decreased with age in AL fed rats, no change occurred in DR rats. Because age-related increases in cholesterol/phospholipid occurred in both AL and DR groups, fluidity loss may be influenced by factors other than cholesterol. We suggest that lipid peroxidation may be a major factor in the change in fluidity during the aging process.

Keywords—Synaptosomes, Membrane fluidity, Free radicals, Hydrogen peroxide, Cholesterol/phospholipid, Age, Dietary restriction

INTRODUCTION

Progressive deterioration of physiological systems and the increasingly deleterious consequences of disease are inherent to the aging process in mammals.[1,2] It has long been known that dietary restriction increases both the mean and maximum length of life of laboratory rodent populations.[3] Indeed, it is the only known manipulation that clearly increases the latter in mammals.[2] Moreover, in recent years, dietary restriction has been shown to retard the rate of age-related physiological deterioration.[4] All these findings strongly indicate that dietary restriction prolongs life by delaying the aging processes.

Many hypotheses have been proposed concerning the mechanism by which dietary restriction prolongs life; most have been rejected on the basis of subsequent experimental findings. The free radical theory of aging proposed by Harman[5] poses the interesting possibility that dietary restriction may exert its anti-aging effect by modulating free radical metabolism. New evidence emerging from studies on dietary restriction and free radical biochemistry strongly supports such interactions.[4,6] Recently, our laboratory clearly documented the link between free radical metabolism and its modulation by dietary restriction by demonstrating the protection of membrane stability [7–10] and the enhancement of cytosolic protective factors during senescence.[11,12] The aim of our study was to determine whether cellular mechanisms underlying these beneficial effects of anti-radical action of dietary intervention also occur in the aging brain.

Brain tissue offers at least four advantages for exploring free radical metabolism in relation to aging. First, the postmitotic neuronal cellularity undergoes no further differentiation. The brain is consequently more vulnerable to such prolonged oxidative stress as free radical damage during aging. Indeed, this is evident from the greater accumulation of lipofuscin or age-pigment in neurons compared to cells undergoing mitosis.[13] Second, brain tissue possesses a high lipid content. Third, consumption of O_2 by the brain tissue is high. The human brain requires one-fourth of the total O_2 intake, making brain tissue one of the most active O_2 respiring organs in a body based on weight. Fourth, the rat brain possesses a relatively weak antioxidant

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defense system.[14,15] In spite of its high O_2 consumption, the brain curiously contains less active superoxide dismutase, catalase and peroxidases than other enzymes.[16] The conclusion from these points is that the brain exhibits a heightened vulnerability to the increased radical activity seen during aging. Thus, it is an excellent model for exploring the effects of interactions between age and nutrition on free radical metabolism and its effect on the interrelations between structural alteration and functional deficit.

Few experimental data are available in the gerontological literature on free radicals and dietary restriction in relation to the latter's anti-aging action on brain, although there is a strong indication that age-related deterioration of such brain functions as behavioral responses[17,18] and receptor activity can be attenuated by dietary restriction.[19] Thus, our aim was to gain information that would delineate the molecular basis for the interrelationships between free radical effect on neuronal membrane structural integrity and functional activities in the aged brain.

MATERIALS AND METHODS

Animals and dietary regimen

Male specific-pathogen free Fischer 344 rats were delivered from Charles River Breeding Lab (Kingston, MA) to our barrier facility at the time of weaning (4 weeks old). Operational procedures of the barrier and detailed information on the diet have been previously published.[20] A semi-synthetic diet prepared by Purina-Ralston (Richmond, IN) was given ad lib until 6 weeks of age, at which time one-half of the rats were randomly selected for our diet restricted (DR) group. The DR rats were allowed only 60% as much food as that consumed by the ad lib (AL) group. Rats were killed by decapitation, the brain tissue immediately excised and stored at −70°C.

Preparation of synaptosomes

Synaptosomal membranes were prepared by the modified method of Jones et al.,[21] utilizing the frontal cortex of rats at 6 and 24 months of age. Pooled forebrains (4.8 g) of three rats were homogenized with 5 strokes at 800 × g in 20 vol of isolation medium, 10% sucrose containing 1.0 mM K-EDTA and 10 mM Tris-HCl (pH 7.5). The homogenates were centrifuged at 1,000 × g for 10 min, and the supernatant was removed and centrifuged at 9,500 × g for 15 min. The pellets were washed once with isolation medium to yield crude mitochondrial fractions, then dissolved with 5.5 ml of isolation medium. For lysis, crude mitochondria were homogenized with 18 ml of 50% sucrose. The lysate was carefully overlaid on two discontinuous sucrose density gradient systems (7 ml of 32% and 8.3 ml of 39%, respectively), and upper phase of total volume of 16.5 ml. These density gradients were centrifuged at 65,000 × g in a Beckman SW-28 swing-out rotor for 100 min (Fig. 1).

Purity of subfractionated synaptosomes

Purity of isolated synaptosomal fraction was tested by two marker enzymes, acetylcholinestrase and 2′,3′-cyclic nucleotide-3′-phosphohydrolase, according to the methods of Ellman et al.[22] and Olafson et al.,[23] respectively.

Measurement of membrane fluidity

The quantitative measurement of membrane fluidity employed the fluorescence polarization technique described by Yu et al.[10] with TMA-DPH (1,4-(trimethylammoniumphenyl)-6-phenyl 1-1,3,5-hexatriene) sensing as a fluorescence probe. Membrane preparations (50 μg protein) were suspended in 50 mM Tris-HCl buffer (pH 7.4), mixed with TMA-DPH prepared from a stock solution of 5 mM TMA-DPH solubilized in tetrahydrofurans, and incubated at 37°C for 30 min. Fluorescence polarization was determined using a Perkin Elmer LS-50 fluorescence spectrophotometer equipped with rotating polarizing filters with samples exposed to a wide range of temperatures (15–40°C). Samples were excited at 360 nm, and emission intensity was read at 435 nm. Calculation of the values for polarization (P) and fluorescence anisotropy (r) of the samples were done by the method of Yu et al.[10]

Determination of reactive oxygen species (ROS)

Determination of the formation of ROS by synaptosomes was carried out according to the fluorescent dye method described by Lebel and Bondy.[24,25] Synaptosomes were incubated with 5 μM DCFH-DA (2′,7′ dichlorofluorescein diacetate, purchased from Molecular Probes, Eugene, OR) for 15 min at 37°C. The excess unbound probe was removed by spinning down the synaptosomes at 12,500 × g for 10 min. Two different conditions were used for the measurement of ROS. One was the determination of ROS under stimulated conditions. The synaptosomes were incubated in the presence of ascorbate (0.1 mM)/$FeSO_4$(5 μM) for 30 min at 37°C. The second condition was to attain the basal level of the generation of reactive species. In this case, the synaptosomes were incubated under similar conditions without ascorbate/$FeSO_4$. In both cases, the fluorescent intensity due to the ROS was measured at

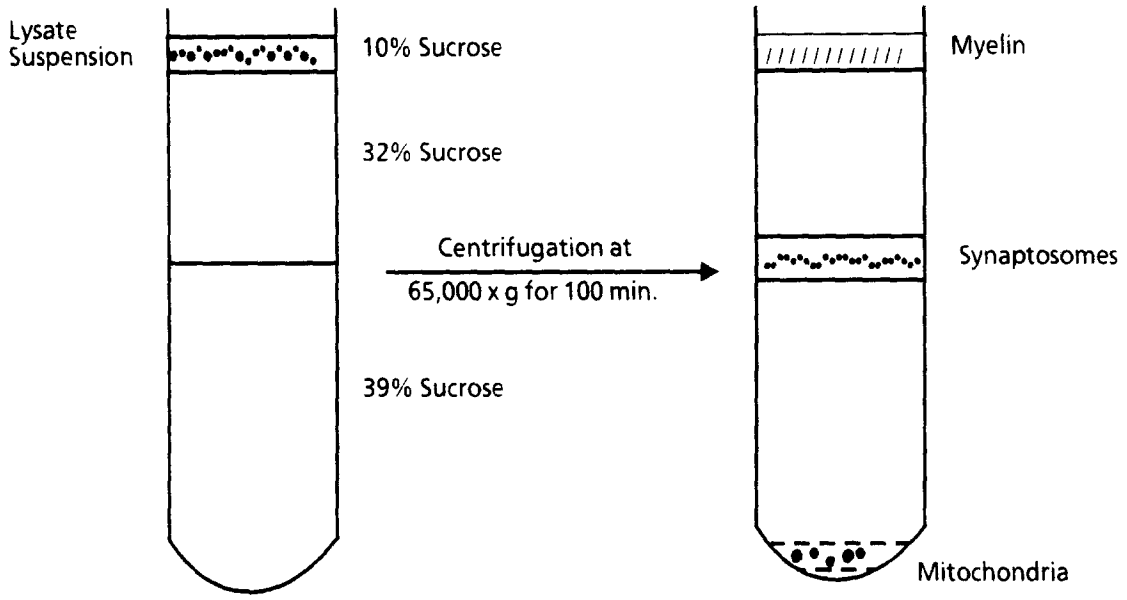

Fig. 1. Schematic presentation of sucrose gradient system for the preparation of synaptosomes.

wavelengths of 488 nm (for excitation) and 528 nm (for emission).

Analysis of cholesterol and phospholipid

Membrane cholesterol and phospholipid content were determined by the method of Laganiere and Yu.[26]

Data analysis

Statistical analysis was done using the statistical package Super ANOVA. A one-factor ANOVA was first used to determine the overall significance of the data. Duncan's multiple regression test was used to determine group differences for age and dietary groups.

RESULTS

The brain weight of rats in both AL and DR groups progressively increased through 24 months (Fig. 2). DR had no effect on brain weight, and the brain weights of DR rats were nearly identical to those of AL rats at all ages. The body weights of the DR rats were approximately 60% of those of the AL groups (data not shown). Therefore, when the brain weight was normalized to body weight, a substantially high ratio was seen in the DR groups (Fig. 3). The yield of synaptosomes isolated from rat brains differed between the two groups. As shown in Figure 4, synaptosomes recovered from the AL rats were significantly fewer than those from DR rats after 12 months of age.

A better characterization of brain subcellular membranous components required the assurance of homogeneity of the isolated membrane preparation. To achieve this goal, a modified discontinuous sucrose density gradient system was developed (Fig. 1). The purity of isolated subfactions from brain homogenates was assessed (Table 1) by the determination of specific activity of two enzymes, acetylcholinesterase for synaptosomes, and 2′,3′-cyclic nucleotide-3′-phospho-hydrolase for myelin fraction. As shown in Table 1, synaptosomal and myelin fractions are reasonably well separated by our modified sucrose density gradient method.

One of the purposes of this study was to investigate the synaptosomal membrane fluidity modulated by

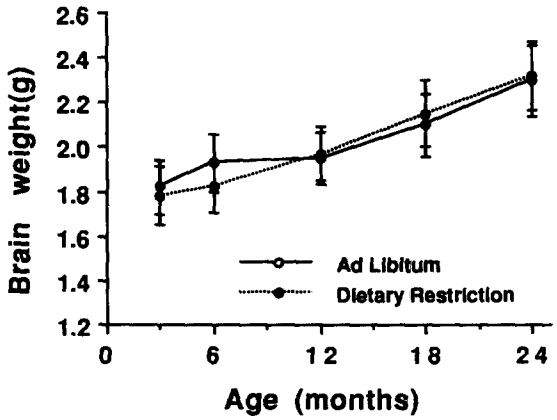

Fig. 2. Age-related changes in brain weight. Values are the mean ± SD of nine rats per group.

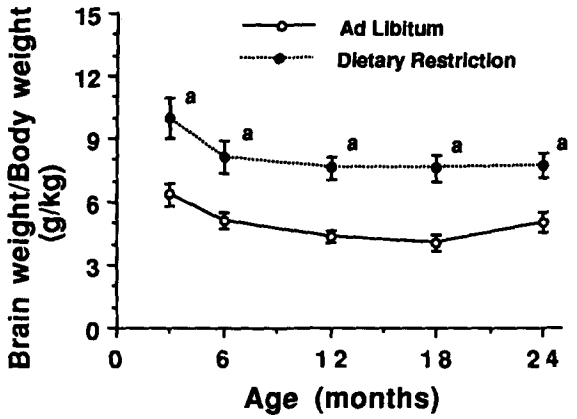

Fig. 3. Data on the ratios of brain weight/body weight of ad lib fed and restricted rats. Values are the mean ± SD of nine rats per group. [a]Significant difference from AL group ($p < 0.01$).

both age and dietary restriction. Our research shows that membrane fluidity was influenced substantially by both parameters. As shown in Figure 5, the membrane fluidity of synaptosomes from 24-month-old AL rats was significantly lower than that from 6-month-old AL rats. These age-dependent decreases were not observed in synaptosomes isolated from rats in the DR group. At both ages, the fluidity of synaptosomes from DR rats was higher than the age-matched AL group. Similar results were obtained when membrane fluidity was assessed by anisotropy (Fig. 6).

Investigations into the generation of reactive oxygen species (ROS) such as superoxide, hydroxyl radicals, and hydrogen peroxide in synaptosomes were carried out under two experimental conditions, one at basal level of the production without stimulation for lipid peroxidation and the second with ascorbate/Fe^{2+}-induced lipid peroxidation. As expected, the production of ROS responded in a different manner.

Table 1. The Marker Enzyme Activities in Brain Subfractions

Subfraction	Acetylcholinesterase*	2′,3′-Cyclic Nucleotide-3′-Phosphohydrolase*
Myelin	1.89 + 0.12	2.37 + 0.15
Synaptosomes	19.27 + 0.76	1.14 + 0.08
Mitochondria (synaptosome-free)	1.54 ± 0.09	0.98 ± 0.07

* μmole/h/mg protein

Under basal conditions, the generation of ROS, as measured by the changes in fluorescence intensity of DFC incorporated in the synaptosomes, increased significantly between 6 and 24 months of age in AL-fed rats, but DR totally prevented the increase (Fig. 7). Stimulation of synaptosomes to lipid peroxidation by ascorbate/Fe^{2+} increased ROS production approximately 5–6-fold over the unstimulated basal values. The increase in ROS production observed during stimulation declined with age in both AL and DR groups (Fig. 8).

Synaptosomal membrane cholesterol was unchanged between 6 and 24 months of age in the AL group. In contrast, membrane cholesterol significantly decreased in DR rats (data not shown). As shown in Table 2, the ratios of cholesterol/phospholipid increased with age in both dietary groups, but no differences were observed between dietary groups at any age.

DISCUSSION

A growing body of evidence has been gathered during the last decade that supports the involvement of

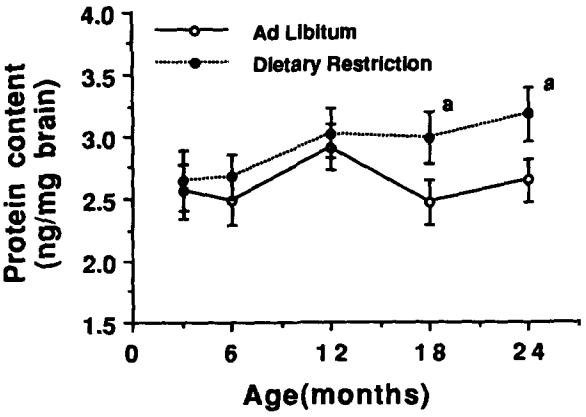

Fig. 4. Data on the yield of synaptosomes isolated ad lib fed and restricted rats. Values are the mean ± SD of nine rats per group. [a]Significant difference from AL group of the same age group ($p < 0.05$).

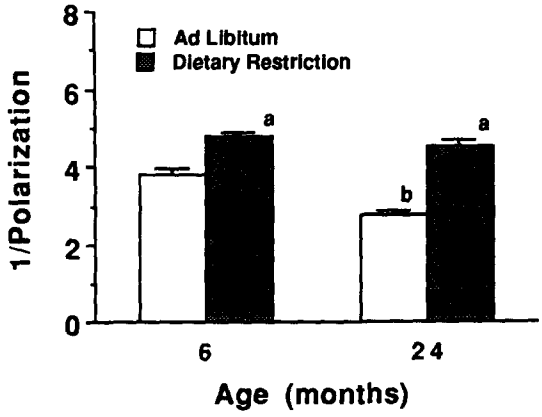

Fig. 5. The effect of age and dietary restriction on membrane fluidity expressed by change in polarization. Values are the mean ± SD of five rats per group. [a]Significant difference from AL group of the same age group ($p < 0.05$).

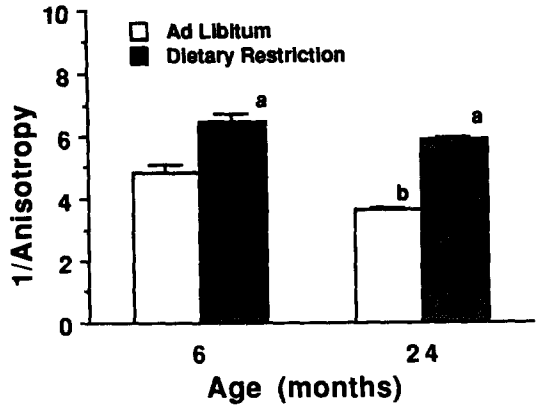

Fig. 6. The effect of age and dietary restriction on membrane fluidity expressed by change in anisotropy. Values are the mean ± SD of five rats per group. [a]Significant difference from AL group ($p < 0.05$). [b]Significant difference from 6 months old AL rats ($p < 0.05$).

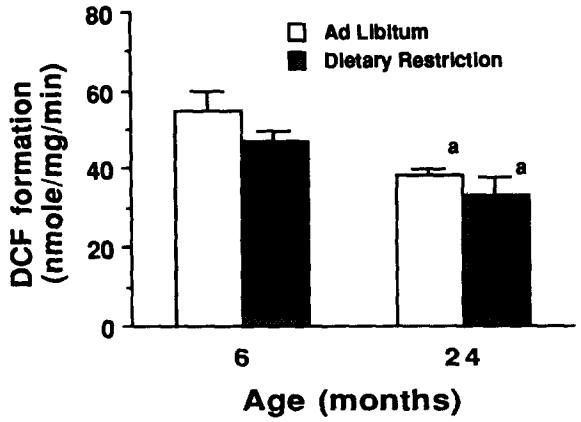

Fig. 8. The formation of ROS at stimulated level by synaptosomes and modulation by dietary restriction. Values are the mean ± SD of five rats per group. [a]Significant difference from 6-month-old rats ($p < 0.05$).

free radicals in the aging and age-related pathologic processes.[3] Accumulation of oxidative damage by free radicals is readily observed in aged tissues.[4,6,27–30] Previous studies showed that age-related changes in synaptosomal membranes were responsible for functional deficits seen in the aged animals. For instance, the alterations in dopamine uptake shown by Maguire and Druse[31] and the GABA(γ-aminobutyric acid) transport by synaptosomes by Strong and Wood[32] were correlated with age-induced changes in synaptosomal fluidity. The damage includes elevated lipid peroxidation as well as alterations in membrane structure and composition.[8,10] More recent experimental data,[33,34] cataloging oxidative damage of DNA and modulation of gene expression, further implicate free radicals in the aging process. The brain, for reasons explained in the Introduction, is especially susceptible to oxidative stress and, therefore, is an ideal organ in which to assess interactions between free radical-induced reactions and the antioxidative action by dietary restriction.

Our findings regarding changes in synaptosomal membrane fluidity are consistent with a general trend of biological alterations that undergo the age-related deterioration.[10,27] One of the salient findings of our current study concerns the possible cellular mechanisms underlying the change in the membrane fluidity. Most studies on the age-related membrane deterioration with an increased membrane rigidity have claimed the increased cholesterol with age in the membrane to be a causal factor responsible for the membrane rigidity during senescence. However, this notion has to be reevaluated in light of our new findings on molar ratio of cholesterol/phospholipid, as shown in Table 2. According to the data in this table, the cholesterol contents were identical in both AL and DR rats, yet the membrane rigidity in the former is significantly increased. Similar findings and conclusions have been made in recent studies on liver mitochondrial and microsomal membranes.[10]

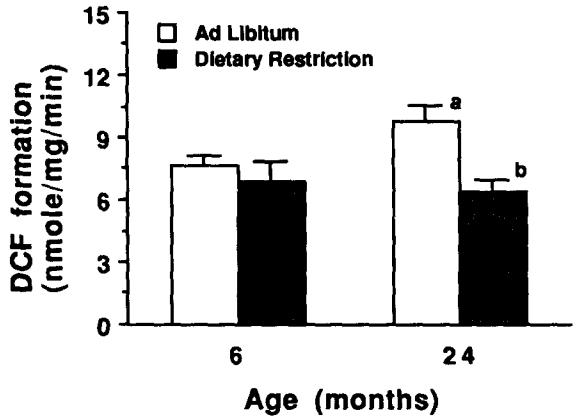

Fig. 7. The formation of ROS at basal level by synaptosomes and modulation by dietary restriction. Values are the mean ± SD of five rats per group. [a]Significant difference from 6-month-old AL rats ($p < 0.05$). [b]Difference from AL rats of the same age group ($p < 0.05$).

Table 2. Ratio of Cholesterol/Phospholipid in Synaptosomal Membranes

Age (months)	AL	DR
3	0.38 ± 0.07	0.37 ± 0.06
6	0.45 ± 0.05	0.43 ± 0.07
12	0.47 ± 0.04	0.45 ± 0.06
18	0.58 ± 0.08	0.55 ± 0.06
24	0.59 ± 0.06	0.57 ± 0.06

n = 9 rats per group.
No significant difference was observed between dietary groups at any age.

One area that could be a major contributing factor for the changes in membrane fluidity is the age-related increase in synaptosomal membrane peroxidation. There are, in fact, convincing data to suggest that membrane rigidity can be readily induced by lipid peroxidation,[10,35] and elevated lipid peroxidation in the aging brain has been well documented.[32,36] The importance of altered membrane lipid structure in the aging brain was highlighted by Schroeder,[37] who noted that changes in membrane lipid symmetry by lipid peroxidation occurred during aging. Several laboratories,[36] including our own,[10] have shown that lipid peroxidation can play a major role in modifying the membrane fluidity by oxidized membrane polyunsaturated fatty acids. The oxidative modifications of polyunsaturated fatty acids generally induce the rigidity of lipid bilayer membrane, due to the steric hindrance restricting the rotational movement of lipid molecules. Such peroxidative deteriorations are expected to occur more readily in the synaptosomes of AL rats for the following reasons. In our study, the synaptosomes isolated from aged AL rats were capable of producing significantly more ROS than those isolated from DR rats, as shown in Figure 8, making the membrane more vulnerable to peroxidation. It is quite feasible, then, that age-related loss in the synaptosomal membrane fluidity is in fact due to oxidative alterations of the membranes in AL rats. Similar conclusions were reached with regard to the age-related membrane rigidity in both mitochondrial and microsomal membranes in the liver.[10]

In summary, the involvement of lipids and membranes in essential brain functions has been well established,[38-40] and numerous neurochemical studies have demonstrated that many of these brain functions deteriorate with age.[41] Brain dysfunction with advanced age may be partly due to the oxidative alterations of neuronal membranes.[42-44] Our evidence suggests that the biochemical basis for age-related deterioration is due to endogenous generation of reactive oxygen species. Equally interesting is the finding that the age-related synaptosomal alterations and ROS production can be attenuated by DR, the only known anti-aging intervention in laboratory rodents. These findings strongly suggest that the anti-aging action of DR may indeed play a pivotal role in attenuating free radical damage on cellular structural integrity.

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REFERENCES

1. Masoro, E. J. Use of rodents as models for the study of "normal aging": Conceptual and practical issues. *Neurobiol. Aging* **12**:639–643; 1991.
2. Weindruch, R.; Walford, R. L. *The retardation of aging and disease by dietary restriction.* Springfield, IL: Charles C. Thomas Pub. Co.; 1988.
3. Porta, E. A. Role of oxidative damage in the aging process. In: Chow, C. K., ed. *Antioxidant defense mechanisms.* Vol. 3. Boca Raton: CRC Press; 1988:1–52.
4. Yu, B. P. Food restriction research: Past and present status. In: Rothstein, M., ed. *Review of biological research in aging.* Vol. 4. New York: Wiley-Liss, Inc.; 1990:349–371.
5. Harman, D. Aging: A theory based on free radical and radiation chemistry. *J. Gerontol.* **11**:298–300; 1956.
6. Habib, M.; Dickerson, F.; Mooradian, A. G. Ethane production rate in vivo is reduced with dietary restriction. *J. Appl. Physiol.* **68**:2588–2590; 1990.
7. Lee, D. W.; Yu, B. P. Modulation of free radicals and superoxide dismutase by age and dietary restriction. *Aging* **2**:357–362 1990.
8. Laganiere, S.; Yu, B. P. Effect of chronic food restriction in aging rats. I. Liver subcellular membranes. *Mech. Age. Dev.* **48**:207–219; 1989.
9. Choi, J. H.; Yu, B. P. Free radical generation and membrane fluidity of aging brain synaptosomes. *Gerontologist* **32**:30; 1992.
10. Yu, B. P.; Suescun, E. A.; Yang, S. Y. Effect of age-related lipid peroxidation on membrane fluidity and phospholipase A2: Modulation by dietary restriction. *Mech. Age. Dev.* **65**:17–33; 1992.
11. Laganiere, S.; Yu, B. P. Effect of chronic food restriction in aging rats. II. Liver cytosolic antioxidants and related enzymes. *Mech. Age. Dev.* **48**:221–230; 1989.
12. Lee, D. W.; Yu, B. P. The age-related alterations in liver microsomal membranes. In: Kitani, K., ed. *Liver and aging.* New York: Elsevier; 1991:17–26.
13. Volicer, L. V.; Crino, B. P. Involvement of free radicals in dementia of the alzheimer type: A hypothesis. *Neurobiol. Aging* **11**:567–571; 1990.
14. Calderini, G.; Bonetti, A. C.; Battistella, A.; Crews, F. T.; Toffano, G. Biochemical changes of rat brain membranes with aging. *Neurochem. Res.* **8**:483–492; 1983.
15. Benzi, G.; Pastoris, O.; Marzatico, F.; Villa, R. F. Age-related effect induced by oxidative stress on the cerebral glutathione system. *Neurochem. Res.* **14**:473–481; 1989.
16. Arai, H.; Kogure, K.; Sugioka, K.; Nakano, M. Importance of two iron-reducing systems in lipid peroxidation of rat brain: Implications for oxygen toxicity in the central nervous system. *Biochem. Int.* **14**:741–749; 1987.
17. Joseph, J. A.; Whitaker, G.; Roth, G. S.; Ingram, D. K. Lifelong dietary restriction affects striatally mediated behavioral responses in aged rats. *Neurobiol. Aging* **4**:191–196; 1983.
18. Ingram, D. K.; Weindruch, R.; Spangler, E. L.; Freeman, J. R.; Walford, R. L. Dietary restriction benefits learning and motor performance of aged mice. *J. Gerontol.* **42**:78–81; 1986.
19. Roth, G. S.; Ingram, D. K.; Joseph, J. A. Delayed loss of striatal dopamine receptors during aging of dietarily restricted rats. *Brain Res.* **300**:27–32; 1984.
20. Yu, B. P.; Masoro, E. J.; McMahan, C. A. Nutritional influences on aging of Fischer 344 rats: I. Physical metabolic and longevity characteristics. *J. Gerontol.* **40**:657–670; 1985.
21. Jones, D. H.; Matus, A. I. Isolation of synaptic plasma membrane from brain by combined flotation-sedimentation density gradient centrifugation. *Biochem. Biophys. Acta* **366**:276–287; 1974.
22. Ellman, G. L.; Courtney, K. D; Andras, V. Jr.; Fetherstone, R. M. A new and rapid colorimetric determination of acethyl cholinesterase activity. *Biochem. Pharmacol.* **7**:88–96; 1961.
23. Olafson, R. W.; Drummond, G. I.; Lee, J. H. Studies on 2′,3′-cyclic nucleotide-3′-phosphohydrolase from brain. *Can. J. Biochem.* **47**:961–966; 1969.
24. Lebel, C. P.; Bondy, S. C. Sensitive and rapid quantitation of oxygen reactive species formation in rat synaptosomes. *Neurochem. Int.* **17**:435–440; 1990.
25. Lebel, C. P.; Odunze, I. N.; Adams, J. D.; Bondy, S. C. Perturbations in cerebral oxygen radical formation and membrane order

following vitamin E deficiency. *Biochem. Biophys. Res. Commu.* **163:**860–866; 1989.
26. Laganiere, S.; Yu, B. P. Modulation of membrane phospholipid fatty acid composition by age and food restriction. *Gerontology* **39:**7–18; 1993.
27. Floyd, R. A.; Zaleska, M. M.; Harmon, H. J. Possible involvement of iron and oxygen free radicals in aspects of aging in brain. In: Armstrong, D.; Sohal, R.; Cutler, R. G.; Slater, T. E., eds. *Free radicals in molecular biology, aging and disease.* New York: Raven Press; 1984:143–161.
28. Zhan, H.; Sun, C. P.; Liu, C. G.; Zhou, J. H. Age-related change of free radical generation in liver and sex glands of rats. *Mech. Age. Dev.* **62:**111–116; 1992.
29. Ando, S.; Kon, K.; Aino, K.; Totani, Y. Increased levels of lipid peroxides in aged rat brain as revealed by direct assay of peroxide values. *Neurosci. Lett.* **113:**199–204; 1990.
30. Cadenas, E.; Varsavsky, A. I.; Boveris, A.; Chance, B. Oxygen- or organic hydroperoxide-induced chemiluminescence of brain and liver homogenates. *Biochem. J.* **198:**645–654; 1981.
31. Maguire, P. A.; Druse, M. J. The influence of cholesterol on synaptic fluidity and dopamine uptake. *Brain Res. Bull.* **22:**431–437; 1989.
32. Strong, R.; Wood, W. G. Membrane properties and aging: In vivo and in vitro effects of ethanol on synaptosomal Γ-aminobutyric acid (GABA) release. *J. Pharmacol. Exp. Ther.* **229:**726–730; 1984.
33. Fraga, C. G.; Shigenage, M. K.; Park, J. W.; Degan, P.; Ames, B. N. Oxidative damage to DNA during aging: 8-hydroxy-2′-deoxyguanosine in rat organ DNA and urine. *Proc. Natl. Acad. Sci. USA* **87:**4533–4537; 1990.
34. Chung, M. H.; Kasai, H.; Nishimura, S.; Yu, B. P. Protection of DNA damage by dietary restriction. *Free Radic. Biol. Med.* **12:**523–525; 1992.
35. Vorbeck, M. L.; Martin, A. P.; Long, J. W.; Smith, J. M.; Orr, R. D. Aging-dependent modification of lipid composition and lipid structural order parameter of hepatic mitochondria. *Arch. Biochem. Biophys.* **217:**351–361; 1982.
36. Viani, P.; Cervato, G.; Fiorilli, A.; Cestaro, B. Age-related differences in synaptosomal peroxidative damage and membrane properties. *J. Neurochem.* **56:**253–258; 1991.
37. Schroeder, F. Role of membrane lipid asymmetry in aging. *Neurobiol. Aging* **5:**323–333; 1984.
38. Govoni, S.; Memo, M.; Saianti, L.; Spano, P. F.; Trabucchi, M. Impairment of brain neurotransmitter receptors in aged rats. *Mech. Age. Dev.* **12:**39–46; 1980.
39. Aprikyan, G. V.; Gekchyan, K. G. Release of neurotransmitter amino acids from rat brain synaptosomes and its regulation in aging. *Gerontology* **34:**35–40; 1988.
40. London, E. D.; Waller, S. B.; Ellis, A. T.; Ingram, D. K. Effects of intermittent feeding on neurochemical markers in aging rat brain. *Neurobiol. Aging* **6:**199–204; 1985.
41. Wood, W. G. Regulation of brain membrane function in aged organisms. In: Davis, B. B.; Wood, W. G., eds. *Homeostatic function and aging.* New York: Raven Press; 1985:125–138.
42. Wood, W. G.; Strong, R.; Williamson, L. S.; Wise, R. W. Changes in lipid composition of cortical synaptosomes from different age groups of mice. *Life Sci.* **35:**1947–1952; 1984.
43. Wheeler, D. D. Aging of the high affinity GABA transporter in synaptosomes from the hypothalamus of the rat. *Exp. Geront.* **18:**125–135; 1983.
44. Bondy, S. C. Reactive oxygen species: Relation to aging and neurotoxic damage. *Neurotoxicology* **13:**87–100; 1992.