Mechanism of Food Restriction: Protection of Cellular Homeostasis (42982)

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Abstract. **The major difficulty in defining the mechanisms for the action of food restriction relates to its diversity and lack of specificity. In searching for the common factor(s) and commonality involved in such diversified effects, a cellular homeostasis mechanism is proposed. A study initiated in our laboratory strongly indicates that the cellular homeostatic mechanism is seriously compromised by oxidative damage of free radical reaction with aging. Data on mitochondrial hydroperoxide, microsomal cytochrome P-450 breakdown, and reduction of cytosolic antioxidant capacity support the notion. Remarkably, food restriction attenuates all of these age-related changes. It is concluded therefore that food restriction preserves the homeostatic regulatory processes by maintaining the integrity of (i) membrane structure and function, (ii) proper redox state of cellular components, and (iii) detoxification process of xenobiotics.** [P.S.E.B.M. 1990, Vol 193]

The cellular homeostasis concept underlines the importance of proper maintenance of various processes in the cell to regulate the constancy of the cellular internal environment. In an oxygen-respiring organism, cellular homeostasis is continuously challenged by the oxidative process of free radicals, and, in an old organism, this threat could be more serious because of the increased free radical activity and deteriorated defense system during aging (1). Therefore, cellular aging is characterized by a progressive functional deficit with a concomitant loss in the ability to withstand both external and internal threat, indicating weakened self-regulating mechanisms responsible for the maintenance of homeostasis.

In formulating a mechanism common to all of the actions of food restriction against a wide spectrum of aging and disease processes, we propose a working hypothesis of homeostasis mechanism by which food restriction slows cellular aging processes by protecting the cellular internal environment. In this article, our preliminary data supporting this hypothesis are presented.

Materials and Methods

Animals and Food Restriction. Specific-pathogen free Fischer 344 male rats (Charles River Laboratories) were used. All rats were fed *ad libitum* until 6 weeks of age, at which time they were separated into two groups: *ad libitum*-fed control and restricted group, whose food intake was restricted to 60% of the food intake of control (2).

Tissue Preparation. Preparation of microsomes from liver and kidney was carried out according to the method of Lagniere and Yu (3). Liver cytosol fraction was prepared according to the method of Wright *et al.* (4).

Determination of H_2O_2. H_2O_2 formation by liver microsomes was measured according to the method of Thurman *et al.* (5). The reaction mixture contained 80 m*M* potassium phosphate buffer (pH 7.6), 10 m*M* $MgCl_2$, 5 m*M* sodium azide, and microsomes (0.5 mg/ml). The reaction was initiated by the addition of NADPH (0.6 m*M*). After removal of precipitate protein by trichloroacetic acid and centrifugation, 0.2 ml of 10 m*M* ferrous ammonium sulfate and subsequently 0.1 ml of 2.5 *M* potassium thiocyanate were added. The absorption of the red ferrithiocyanate complex formed by H_2O_2 was read at 480 nm.

Measurement of Cytochrome P-450. Degradation of cytochrome P-450 by lipid peroxidation during incubation was measured according to the method of Schacter *et al.* (6). The reaction was induced by NADPH and ADP iron. The P-450 content of the incubation medium was measured by the differences in absorbancy between 450 and 490 nm.

Measurement of Cytosolic Antioxidant Activity. Estimation of cytosolic inhibitory activity on cytochrome P-450 degradation was carried out with an incubation system containing ADP-iron (5 m*M*/0.2 m*M*), NADPH (1 m*M*), and microsomes (1.0 mg/ml) in 0.1 *M* potassium phosphate buffer (pH 7.4). Aliquots of cytosols isolated from various liver homogenates

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were added to the incubation system. The inhibitory activity was measured by monitoring the extent of inhibition of cytochrome P-450 breakdown 5 min after incubation.

Determination of Fatty Acid Composition. Membrane fatty acids were analyzed according to the method of Laganiere and Yu (7). Peroxidizability index was calculated as percentages of monoenoic, dienoic, trienoic, tetraenoic, pentaenoic, hexaenoic acids in the lipid multiplied by 0.025, 1, 4, 6, and 8, respectively, according to the method of Witting and Horwitt (8).

Results

The alteration in structure and composition of microsomal membranes was evaluated by analysis of fatty acid composition. As shown in Figure 1, peroxidizability of microsomal membrane was much higher in *ad libitum*-fed rats, whereas in food-restricted rats, the peroxidizability was attenuated at much lower levels.

H_2O_2 generation in microsomes was investigated (Fig. 2). Although there were no age-related increases in H_2O_2 formation, microsomes from fully fed control rats consistently produced more H_2O_2 than restricted rats.

Figure 3 shows the inhibitory activity in cytosolic fractions. The suppression of lipid peroxidation by cytosolic aliquots from *ad libitum*-fed and restricted rats were compared by measuring the inhibition of cytochrome P-450 destruction. Cytosols from food-restricted rats exhibited much stronger suppressive activity at all three ages tested than those of the *ad libitum*-fed group.

Degradation of microsomal cytochrome P-450 was studied by incubating microsomes for the condition identical to *in vitro* lipid peroxidation (Fig. 4), and the extent of degradation was monitored by peak disappearance at 450 nm of cytochrome P-450-CO complex.

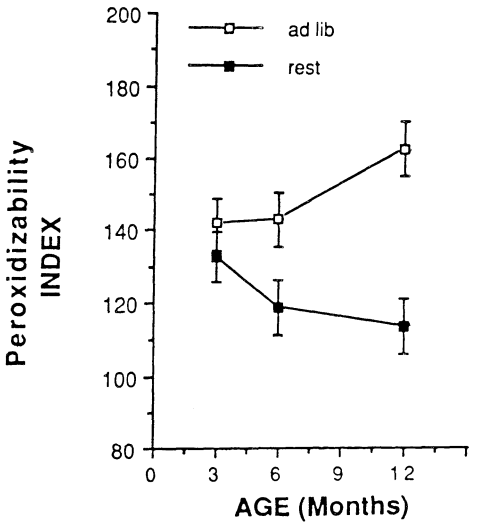

Figure 1. Peroxidizability of kidney microsome fatty acids peroxidizability index was calculated according to the method of Witting and Horwitt (7) as described in Materials and Methods.

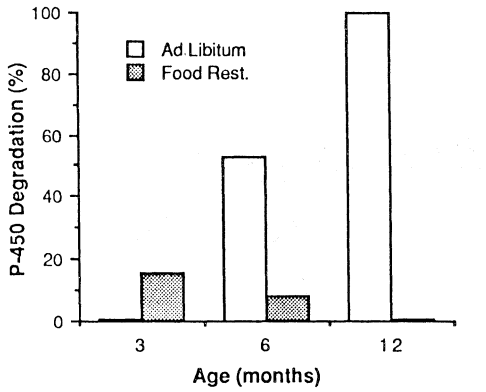

Figure 2. H_2O_2formation in liver microsomes during incubation for 15 min at 37°C. The red ferrithiocyanate complex formed by H_2O_2 was measured at 480 nm.

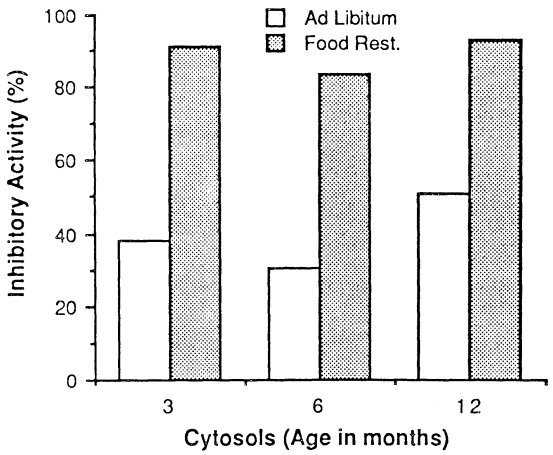

Figure 3. Liver cytosolic inhibitory activity against cytochrome P-450 degradation. Aliquots of cytosols (0.25 mg protein/ml of reaction mixture) were added to the lipid peroxidation incubation system.

The destruction of cytochrome P-450 was age dependent in *ad libitum*-fed rats, and by 12 months of age, the destruction increased to almost 100% within 10 min. Remarkably, very little cytochrome P-450 destruction had taken place in microsomes from restricted rats.

Discussion

Our hypothesis states that food restriction slows the aging process by protecting the self-regulatory mechanisms maintaining cellular homeostasis. This hypothesis is based on the idea that the maintenance of cellular homeostatic environment is possible only when key cellular components and processes are preserved. In this study, we were able to obtain several lines of evidence indicating that those cellular structures and processes are damaged during aging.

As the first evidence on membrane alteration with age, we showed that lipid structure of the microsomal membrane undergoes substantial changes by becoming highly peroxidizable as indicated by the increased peroxidizability index. Consistent with the data is the H_2O_2 production in microsomal membranes as shown in Figure 2. Microsomal membranes from control rats were capable of more H_2O_2 production than that of

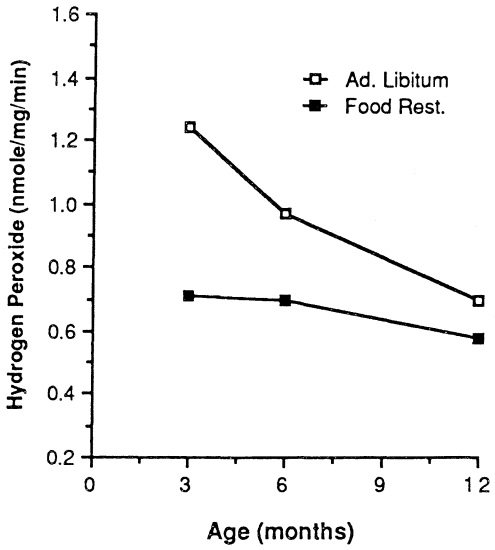

Figure 4. Destruction of cytochrome P-450 during lipid peroxidation. Destruction of cytochrome P-450 was monitored by measuring spectral difference of cytochrome P-450-CO complex at 490 and 450 nm.

restricted rats. In our early study (7), liver mitochondrial and microsomal membranes from *ad libitum*-fed rats contained a higher amount of lipid hydroperoxide than restricted rats. These high peroxide levels could be related to the increased peroxidizability of membrane lipids found in the present study. What is remarkable in the change in membranes was that food restriction effectively managed to remain at the lower levels of all three parameters studied— peroxidizability, H_2O_2 production, and lipid hydroperoxides.

The second line of evidence is the weakened cytosolic defense activity against oxidative process during aging. The antioxidant activity of cytosols is considered part of the key defense system needed for the safeguard of cellular homeostasis. Thus, the age-related deterioration of cytosolic antioxidant activity could create an intolerable situation leading to further membrane instability, redox imbalance, and the inactivation of many enzymes. As shown in this study (Fig. 3), the protection activity in cytosols from liver of food-restricted rats against peroxidation is well preserved, although what factor or factors are responsible for the antioxidant reaction are not completely understood. Data from our laboratory (9) revealed that the levels of reduced glutathione, catalase, and reduced glutathione peroxidase activity are all elevated in food-restricted rats. Koizumi *et al.* (10) reported a similar observation on catalase activity. Therefore, it is reasonable to expect that the internal milieu of the cell is well protected against oxidative damage in food-restricted rats.

The third line of evidence as a possible cause of disruption in cellular homeostasis comes from the changes in microsomal cytochrome P-450. Our data show that microsomal cytochrome P-450 of *ad libitum*-fed rats liver has a progressive age-related breakdown reflecting fragility or instability of microsomal membrane. Since cytochrome P-450 plays a pivotal role in detoxification process of many xenobiotics, it is therefore expected that this progressive breakdown of cytochrome P-450 may cause serious deficits on the ability of the cell to neutralize or eliminate many xenobiotics and metabolic by-products that are known to increase during senescence (11). Interestingly, age-related cytochrome P-450 destruction was clearly dampened by food restriction boosting its active detoxification process, thereby aiding the maintenance of cellular homeostasis.

Putting the data together, it seems evident that cellular homeostasis is seriously challenged by oxidative threat during the course of aging leading to the cell's loss of self-regulating mechanisms vital to its survival. Recent findings on age-dependent deterioration in gene expression of superoxide dimutase and catalase (12) and age-related loss in DNA repair in hepatocytes (13), all seem to support the disrupted homeostatic mechanism.

Our present findings are consistent with the hypothesis that the cellular mechanism by which food restriction protects the self-regulating mechanism is related to the ability of the cells to provide better coordination and integration of components or processes involved in the preservation of the cellular homeostasis.

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