Retardation of the Aging Processes in Rats by Food Restriction

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Restricting the food intake of rodents has long been known to extend the life span, both the mean and the maximum length of life.[1] Questions that have been addressed in recent years and are still under investigation are: Does food restriction retard the primary aging processes? Are most age-associated processes influenced? What are the mechanisms underlying these actions? Findings obtained to date on these questions are the subject of this paper. Particular emphasis will be placed on the studies carried out in our laboratory.

Physiological Systems

Our initial reason for studying the influence of food restriction on age-changes in the physiological systems was our belief that this information would provide insights on mechanism of action. The male Fischer 344 rat was the animal model we used. Rats were singly housed to permit an accurate assessment of food intake and were kept in a barrier facility to maintain their specific pathogen-free status. The food restricted rats were provided 60% of the mean food intake of the rats allowed to eat ad libitum and unless otherwise stated food restriction was initiated at 6 weeks of age (2 weeks postweaning). A semisynthetic diet was used.[2]

Food restriction was found to delay and/or blunt most age-changes in the physiological systems. The data of Liepa *et al.*[3] on plasma cholesterol levels (FIG. 1) are a typical example. At 6 months of age, food restricted and ad libitum fed rats had similar plasma cholesterol levels. In ad libitum fed rats, plasma cholesterol levels were markedly higher by 12 months of age reaching a peak concentration by 18 months of age. In contrast, in food restricted rats an increase in plasma cholesterol level did not occur until 18 months of age and the magnitude of the age-related increase was less marked than in ad libitum fed rats. We have observed preventive effects of food restriction with these male Fischer 344 rats in regard to many other age-changes[1] such as the increase in plasma triglyceride concentration, the loss in lipolytic response of adipocytes to hormones, alterations in skeletal muscle structure and function, the increase in serum calcitonin levels, the decline in spontaneous locomotor activity. The studies of other investigators involving many different mouse and rat strains have yielded similar results; examples of the retardation of age-changes include its effects on central nervous system neurotransmitter receptors and neurochemical markers, lens crystallins, female reproductive function, and immune functions to name a few.

The breadth of these effects on age-changes in the physiological systems provides strong support for the view that food restriction retards fundamental primary aging processes. On the other hand, this breadth undermines the usefulness

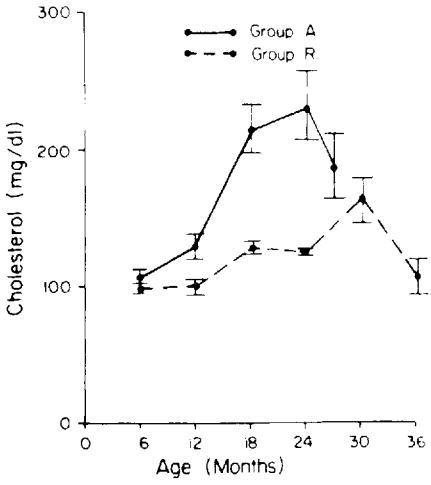

FIGURE 1. Influence of age and food restriction on serum cholesterol concentrations. Group A were ad libitum fed and Group R were fed 60% of the mean food intake of Group A. (From Liepa *et al.*[3] Reprinted by permission from the *American Journal of Physiology*.)

of these actions of food restriction on physiological processes as tools for uncovering the nature of underlying mechanisms.

Disease Processes

The occurrence of age-associated diseases appears to be an almost inevitable consequence of aging. Studies with many different mouse and rat strains have shown that food restriction retards most of these disease processes.[1]

The major age-associated disease processes in male Fischer 344 rats are nephropathy, cardiomyopathy and neoplastic disease. Food restriction was found to influence all three classes of disease processes.[4]

The progression in severity of nephropathy was assessed by sacrificing ad libitum fed and food restricted rats at various ages and examining the kidneys histologically (FIG. 2). The severity of lesions was graded 0 (no lesions), 1, 2, 3, 4, E (end-stage) in the order of increasing severity. The increase in severity with age was significantly greater for ad libitum fed than for food restricted rats.[4] The findings at the time of spontaneous death (TABLE 1) are in accord with this conclu-

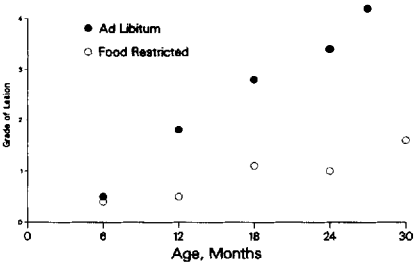

FIGURE 2. Age and the severity of nephropathy. Each point in the graph is the mean value from the analysis of 10 rats. Data on which this graph is based were reported by Maeda *et al.*[4] in which the severity of lesions was assessed using multiple ordered categories and the progression in sacrificed rats analyzed using ridit analysis.

TABLE 1. Severity of Chronic Nephrophy at the Time of Spontaneous Death[a]

Dietary Regimen	Number of Rats Examined	% of Rats with Lesions of Grade:					
		0	1	2	3	4	E
Ad libitum fed	182	0	4	14	14	23	45
Food restricted	145	6	72	15	6	0	1

[a] Data in table are from Maeda *et al.*[4] and Masoro *et al.*[5]

sion.[4,5] At the time of spontaneous death 68% of the ad libitum fed rats had Grade 4 or E lesions compared to 1% of the food restricted rats and 4% of the ad libitum fed rats had Grade 0 and 1 lesions compared to 78% of the food restricted rats. These findings are even more striking when it is also recognized that the food restricted rats are much older at the time of spontaneous death than the ad libitum fed rats.[6]

The progression in severity of cardiomyopathy was also assessed by sacrificing ad libitum fed and food restricted rats at various ages and examining the heart histologically (FIG. 3). The severity of lesions was graded 0 (no lesions) 1, 2, 3 in the order of increasing severity. The increase in severity with age was significantly greater for ad libitum fed than for food restricted rats.[4] The findings at the time of spontaneous death (TABLE 2) agree with this conclusion.[4,5] At the time of spontaneous death 19% of the ad libitum fed rats had Grade 3 lesions compared to 6% of the food restricted rats while 8% of the ad libitum fed rats had no lesions (Grade 0) compared to 21% of the food restricted rats.

The percentage of rats with neoplastic disease at the time of spontaneous death was greater for food restricted than for ad libitum fed rats.[4,5] However, this finding may be due to the fact that food restricted rats live to much older ages than ad libitum fed rats. To assess this possibility, theoretical survival curves (FIGS. 4 and 5) were generated based on all neoplastic diseases or leukemia/lymphoma (a major neoplastic disease in this rat strain) as the sole cause of death. The results show that food restriction delays the occurrence of death due to neoplasia in general or specifically to leukemia/lymphoma to older ages.

Disease and the Physiological Systems

The marked influence on the age-associated diseases raises the possibility that the action of food restriction on the age-changes in physiological systems may be

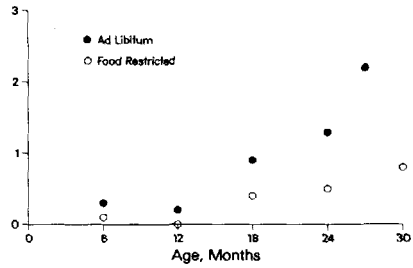

FIGURE 3. Age and the severity of cardiomyopathy. Each point in the graph is the mean value from the analysis of 10 rats. Data on which this graph is based were reported by Maeda *et al.*[4] in which the severity of lesions was assessed using multiple ordered categories and the progression in sacrificed rats analyzed using ridit analysis.

TABLE 2. Severity of Cardiomyopathy at the Time of Spontaneous Death[a]

Dietary Regimen	Number of Rats Examined	% of Rats with Lesions of Grade: 0	1	2	3
Ad libitum fed	182	8	33	40	19
Food restricted	145	21	54	19	6

[a] Data in table are from Maeda *et al.*[4] and Masoro *et al.*[5]

secondary to the retardation of disease. Assessment of the broad spectrum of data collected in our laboratory on the male Fischer 344 rat indicates that usually such is not the case. Specifically, the time course of age-change in the physiological process is usually dissociated from that of the progression or occurrence of disease. For example, the increase in serum cholesterol concentration starts by 12 months of age with maximum levels being reached by 18 months of age in ad libitum fed rats; in these rats severe nephropathy or cardiomyopathy is rarely seen until well after 18 months of age nor is there evidence of an appreciable amount of neoplastic disease by 18 months of age.[4]

However, some of the actions of food restriction on age-changes in physiological process do appear to be secondary to its effect on disease processes. A case in point is the age-associated increase in serum parathyroid hormone concentration in male Fischer 344 rats. In FIGURE 6, age-changes in serum parathyroid hormone concentrations are compared for male Fischer 344 rats fed ad libitum either the usual casein-containing diet or a similar diet in which soy protein has replaced casein and for food restricted rats.[4] Both food restriction and replacing casein with soy protein in ad libitum fed rats almost totally prevented the age-associated increase in serum parathyroid hormone concentration. These two dietary manipulations also retard the progression of nephropathy.[4,8] These findings suggest that at least in part the blunting by food restriction of the age-associated increase in serum parathyroid hormone is secondary to the retardation of kidney disease.

Primary Aging Processes

The findings obtained in our laboratory as well as the research of others strongly indicate that one or more of the primary aging processes is or are major site(s) of action of food restriction. This conclusion is based on: 1) Food restric-

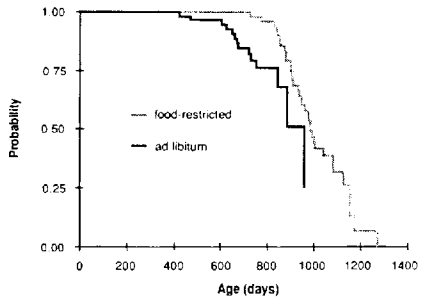

FIGURE 4. Survival curves of ad libitum fed and food restricted rats if neoplastic disease were the sole cause of death. Curves were generated from the data reported by Masoro *et al.*[5] by the Kaplan-Meier method.

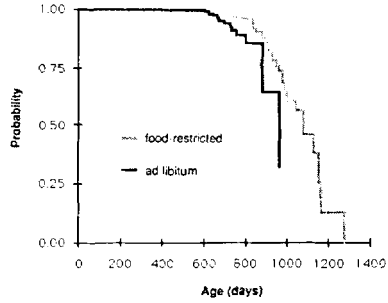

FIGURE 5. Survival curves of ad libitum fed and food restricted rats if leukemia/lymphomas were the sole cause of death. Curves were generated from the data reported by Masoro *et al.*[5] by the Kaplan-Meier method.

tion extends the maximum life span of many different strains of mice and rats. 2) Food restriction retards most age-changes in the physiological systems of rodents indicating an action that is general rather than specifically related to a particular physiological process. 3) Food restriction retards almost all age-associated diseases indicating an action which modulates disease processes in general rather than a specific pathogenesis.

If food restriction acts on the primary aging processes, what is the nature of these processes and how does food restriction influence them? Our recent efforts and those of many other investigators have focused on these two questions.

Mechanism of Action

Specific Nutrient

The possibility that the restriction of a specific nutrient underlies the action of food restriction was investigated in our laboratory. In several of our studies the effects on longevity of restricting a class of nutrients without restricting caloric intake were measured. The results of restricting the mineral component by 40% or the fat component by 40% on survival characteristics are shown in FIGURES 7 and 8. Restriction of neither the minerals nor the fat influenced the life span.[8]

However, restricting the protein component by 40% without restricting caloric intake did increase the median length of life, the age of the tenth percentile

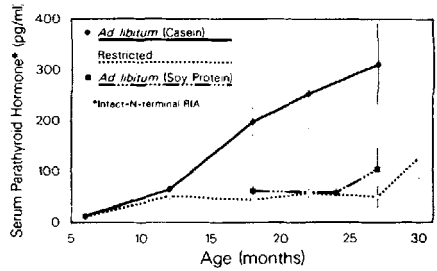

FIGURE 6. Effects of aging and dietary manipulation on serum parathyroid hormone concentration. (From Kalu *et al.*[7] Reprinted by permission from *Endocrinology*.)

survivors and the maximum length of life[6] but much less markedly than when protein restriction was accompanied by a similar reduction in caloric intake (TABLE 3). Moreover, a 40% restriction of caloric intake without restricting protein intake was as effective as caloric restriction which included protein restriction in increasing the median length of life, the age of tenth percentile survivors and the maximum length of life. Our conclusion from these studies is that protein restriction is not a factor in most of the actions of food restriction including its effects on longevity. That protein restriction without caloric restriction decreases the extent of severe nephropathy is probably the major reason for the small increase in longevity. However, caloric restriction with or without protein restriction almost totally prevents the occurrence of severe nephropathy and it is probably for this reason that the level of protein intake has little effect on the longevity of calorically restricted rats.

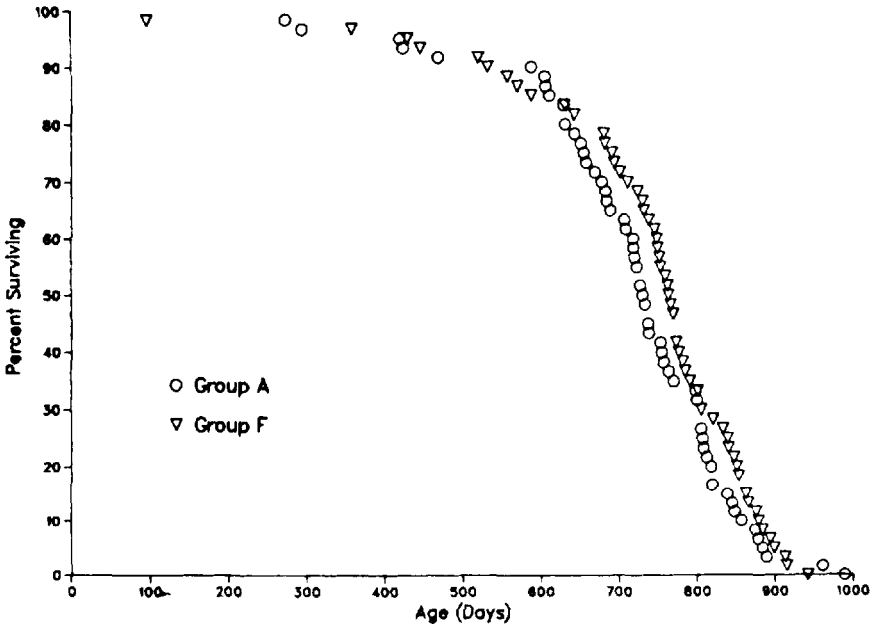

FIGURE 7. Survival curves for rats fed the standard semisynthetic diet (Group A) and rats fed a diet restricting mineral intake by 40% (Group F). The caloric intake of both groups was the same. (From Iwasaki *et al.*[9] Reprinted by permission from the *Journal of Gerontology: Biological Sciences.*)

Thus, our research strongly indicates that the retardation of the aging processes in food restriction is due to the restriction of energy intake rather than a specific nutrient. However, the design of our studies does not completely rule out a possible specific role for the carbohydrate component in this regard.

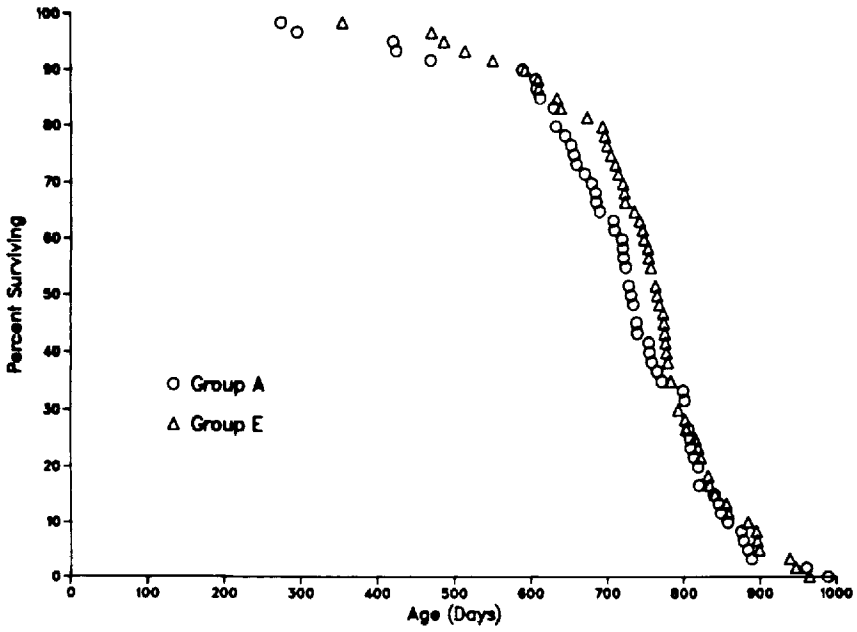

FIGURE 8. Survival curves for rats fed the standard semisynthetic diet (Group A) and rats fed a diet restricting fat intake by 40% (Group E). The caloric intake of both groups was the same. (From Iwasaki *et al.*[9] Reprinted by permission from the *Journal of Gerontology: Biological Sciences*.)

Growth and Development

In 1935, McCay *et al.*[10] postulated that food restriction extends the length of life by slowing growth and development. We directly tested this hypothesis in studies with rats on the following dietary programs:[6,11] Group 1, fed ad libitum throughout life; Group 2, food restricted starting at 6 weeks of age; Group 3, food

TABLE 3. Influence of Caloric Restriction, Protein Restriction and Protein Plus Caloric Restriction on Longevity

% Restriction of Calories	% Restriction of Protein	Median Length of Life Days	Age of 10th Percentile Survivors Days	Maximum Length of Life
0	0	701[a]	822[a]	941[a]
0	40	810[a]	935[a]	969[a]
40	0	956[b]	1158[b]	1295[b]
40	40	936[b]	1121[b]	1275[b]

[a] Data from Yu *et al.*[6]
[b] Data from Masoro *et al.*[5]

restricted from 6 weeks to 6 months of age and thence ad libitum fed; Group 4, food restricted starting at 6 months of age. The results in regard to longevity are summarized in FIGURE 9. Starting food restriction at 6 months of age was found to be as effective as starting at 6 weeks of age in extending the age of the tenth percentile survivors and the maximum length of life. Thus, food restriction is quite effective in the mature rats in which growth is almost complete. Our results are not in accord with the growth and development hypothesis of McCay and his associates.

Body Fat

Food restricted rats have a lower fat content per gram body weight than ad libitum fed rats.[4] It has been proposed that the reduction in body fat contents plays an important role in the life span extending action of food restriction.[12] This hypothesis was tested in our laboratory in studies with the male Fischer 344 rat. The body fat content of ad libitum fed rat was not found to correlate with the length of life and in the case of the food restricted rats there was positive correlation. These findings make it unlikely that reducing the body fat content plays a causal role in the life span extending action of food restriction.

Metabolic Rate

Based on the work of Rubner[13] and his own studies,[14] Pearl postulated that the rate of aging is inversely related to the metabolic rate. Extending this concept, Sacher[15] proposed that food restriction brings about an increase in life span and a slowing of the aging processes by decreasing the metabolic rate. It should be noted that Rubner, Pearl and Sacher viewed metabolic rate in terms of the rate of energy expenditure per unit of body mass.

Our findings with ad libitum fed and food restricted male Fischer 344 rats are not in accord with the hypothesis of Sacher. The data on food intake first alerted us to this. Although food intake per gram body weight decreased by 40% when food intake was reduced by 40%, the changes in body weight in response to food restriction soon resulted in a slightly higher food intake per gram body weight in food restricted than in ad libitum fed rats; these findings were graphically summarized by Masoro[17] in FIGURE 10. This issue was pursued further by McCarter and his associated[18,19] who measured 24 hour oxygen consumption by these rats under usual living conditions. They expressed the data as Kcalories of energy expenditure per Kgram lean body mass per day. Initially, food restriction caused a fall in energy expenditure per unit lean body mass but within 6 weeks of the initiation of food restriction the food restricted and the ad libitum fed rats had similar rates of oxygen consumption per unit of lean body mass. The reason for these findings is that lean body mass is rapidly reduced in proportion to the reduction in energy intake.

These findings make it unlikely that the effects of food restriction are due to a reduction in the intensity of metabolism or to a decreased intake of energy or any other nutrient per unit of lean body mass. Rather they indicate that the antiaging actions of food restriction involve a total organism response possibly involving the nervous system or endocrine system or both.

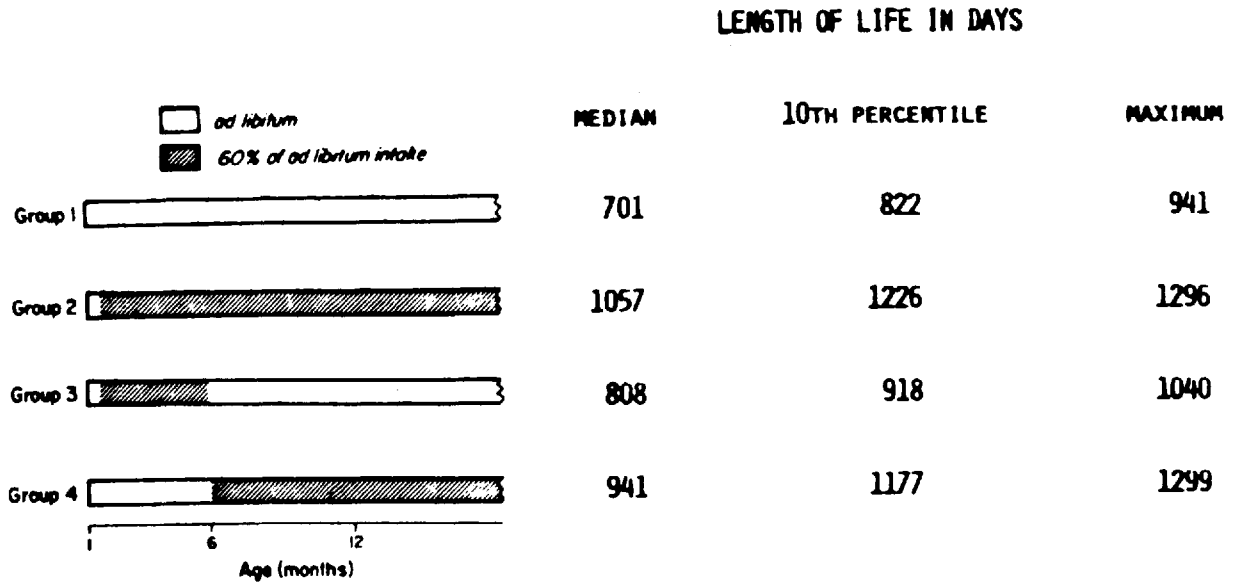

FIGURE 9. The influence of the time of initiation and duration of food restriction (40% reduction in food intake) on longevity. (Data from Yu *et al.*[6] Figure from Masoro.[11] Reprinted by permission from MTP Press Limited.)

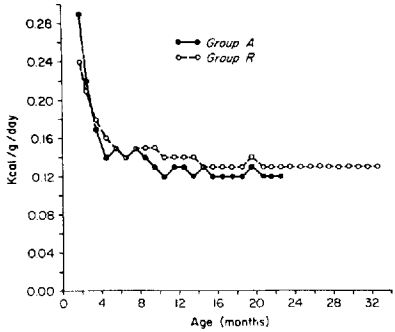

FIGURE 10. Food intake per unit of body mass by ad libitum fed (Group A) and food restricted rats. (Data from Masoro *et al.*[16] Figure from Masoro.[17] Reprinted by permission from Van Nostrand Reinhold.)

Glucocorticoid Cascade Hypothesis

In considering the specific systems that might be involved, the Glucocorticoid Cascade Hypothesis of Aging proposed by Sapolsky *et al.*[20] attracted our attention. This hypothesis is based on the concept that hippocampal neurons rich in glucocorticoid receptors are involved in the negative feedback regulation of plasma glucocorticoid concentrations. Increases in plasma glucocorticoid concentration in response to stress are perceived as downregulating these neuron receptors and when coupled with another insult such as ischemia are believed to result in a loss of these neurons. This hypothesis proposes that by this mechanism there is a gradual loss with advancing age of these hippocampal neurons and the ultimate result is a feed-forward cascade of sustained hyperadrenocorticism. Sapolsky *et al.* further suggested that many of the major detrimental aspects of aging, *e.g.*, immunosuppression, osteoporosis, impaired cognition and many others, are at least in part due to this hyperadrenocorticism.

Studies are being conducted in our laboratory with ad libitum fed and food restricted male Fischer 344 rats to further evaluate the concept of Sapolsky *et al.* and to explore the possibility that food restriction retards the aging processes by preventing the occurrence of hyperadrenocorticism. The following are being assessed in a longitudinal study: the circadian pattern of plasma corticosterone concentration, the plasma concentration of corticosterone binding globulin (CBG), the mean 24-hour concentration of plasma total corticosterone and free corticosterone. In addition, in a cross-sectional study, the rise in plasma corticosterone concentration in response to restraint stress and its recovery following the stress are being assessed.

The basic characteristics of the circadian plasma corticosterone concentration pattern did not change with age through 25 months of age, the oldest rats studied to date. The mean 24-hour plasma total corticosterone concentration in ad libitum fed rats remained at about 100 ng/ml through 13 months of age; by 15 months of age it increased to 130 ng/ml and remained at that level through 25 months of age. Food restricted rats had mean 24-hour plasma total corticosterone concentrations of about 100 ng/ml through 25 months of age. Plasma CBG concentrations remained at about 1500 nM through 25 months of age in ad libitum fed rats but progressively fell in food restricted from about 1500 nM at 3 to 7 months of age to about 850 nM at 21 to 25 months of age. Over most of the 25 months, the plasma free corticosterone was significantly higher in food restricted rats than in ad libitum fed rats; *e.g.*, in the 21 to 25 month age range the concentration in the food restricted rats was twice that of the ad libitum fed rats.

The rise in plasma corticosterone concentration in response to restraint stress and its recovery following the stress were similar in 5 to 6 months old ad libitum fed and food restricted rats. In both rat groups there was a slower recovery of plasma corticosterone concentration following a restraint stress at 18 to 19 months than at 5 to 6 months of age but no further slowing was observed at 23 to 24 months of age.

A conclusion to be drawn from our findings is that food restriction does not retard the aging processes by preventing the occurrence of hyperadrenocorticism. Rather our findings raise the possibility that increased plasma free corticosterone levels might be a factor in the antiaging actions of food restriction. Further research is required to explore this possibility.

Moreover, in the ad libitum fed rats neither a progressive increase in mean 24 hour plasma corticosterone levels or a progressive slowing in recovery of plasma corticosterone following a restraint stress occurred with advancing age. These findings do not support the Glucocorticoid Cascade Hypothesis as describing a major aspect of aging.

Glycation Theory

The proposal by Cerami[21] that glucose may serve as a mediator of aging pointed to another possible mechanism by which food restriction might influence the aging processes. Research aimed at exploring this possibility is in progress in our laboratory.

Cerami believes that the nonenzymatic glycation of proteins and nucleic acids by glucose may underlie many aspects of aging. The initial reaction is between the aldehyde group of glucose and amino groups of the protein or nucleic acids to form a Schiff base. A series of further reactions occur between the glucose adduct and the macromolecule resulting ultimately in what Cerami calls Advanced Glycation End-Products (AGE). Cerami points out that excessive glycation of proteins and nucleic acid may have detrimental consequences such as the loss of enzymatic activity, altered genetic expression, altered binding of regulatory molecules and inappropriate cross-linking of proteins. It is by these effects that it is postulated that glycation mediates aging.

The extent of glycation of macromolecules relates to many factors. The concentration of glucose (or other sugar) in the environment of the macromolecule and the length of time exposed to this concentration are two of the most important.

The influence of food restriction on sustained levels of plasma glucose is being studied in our laboratory with the male Fischer 344 rat. The diurnal pattern of plasma glucose concentration is shown in FIGURE 11 for ad libitum fed and food restricted rats at 4 to 6 months of age. Except for immediately after feeding the food restricted rats had a significantly lower plasma glucose level than ad libitum fed rats. In a longitudinal study the mean 24 hour plasma glucose concentrations have been determined for ad libitum fed and food restricted rats from 3 to 25 months of age. Through this time period food restricted rats have had sustained mean 24 hour plasma glucose levels approximately 15 mg/dl below that of the ad libitum fed rats.

These findings are consistent with the Glycation Theory of Aging but, of course, further work is needed to establish a causal role for the reduced plasma glucose levels in the action of food restriction on the aging processes. Moreover, a reduction in plasma glucose concentration could influence the aging processes by

means other than glycation. It is striking, however, that the rate of glucose utilization per unit lean body mass of food restricted rats is as great as in ad libitum fed rats. This suggests that either glucose effectiveness or insulin sensitivity as defined by Bergman[23] or both is/are increased by food restriction. Whichever be the case this effect of food restriction may be a fundamental mechanism since it permits the effective use of an important but potentially toxic fuel at sustained lower concentrations and thus presumably at less damaging concentrations.

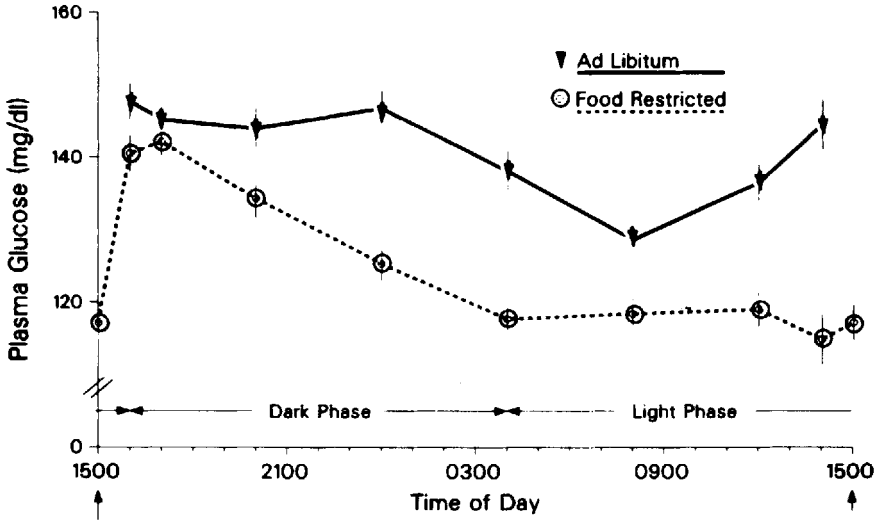

FIGURE 11. Diurnal pattern of plasma glucose concentration in ad libitum fed and food restricted rats in the age range of 4 to 6 months. The *arrow* indicates the time of feeding of the food restricted rats. (From Masoro *et al.*[22] Reprinted by permission from the *Journal of Gerontology: Biological Sciences.*)

Free Radical Theory

Since the free radical theory of aging was proposed in 1956 by Harman,[24] many attempts have been made to relate free radical metabolism to the aging processes. Interventions using antioxidants, free radical scavenging drugs and other agents of this type have been only marginally successful in extending the median length of life and without effect on the maximum life span. This failure does not provide strong evidence against the free radical theory for two reasons: 1) the exogenously administered antioxidant or other drugs may not be distributed to the cellular site of production of the free radicals, and 2) particular antioxidants and other drugs are specific for a particular free radical species and thus are unlikely to have a global effect.

Recently, studies on the effects of food restriction have given new life to the free radical theory. In our laboratory, food restriction of male Fischer 344 rats has been found to modulate free radical production, scavenging enzyme activities, free radical damage and the detoxification of the products of free radical damage.

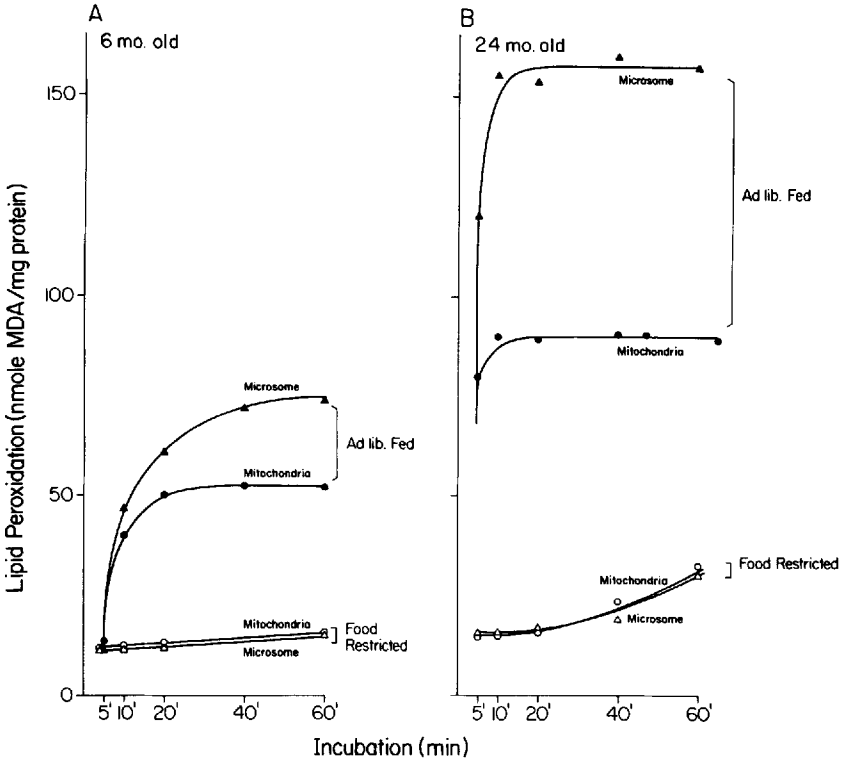

FIGURE 12. *In vitro* enzyme-dependent lipid peroxidation by hepatic membranes. **(A)** 6-month-old rats. **(B)** 24-month-old rats. Peroxidation was performed in the presence of 0.2 mM $FeSO_4$, 5 mM ADP and 1 mM NADPH. (From Laganiere and Yu[25] Reprinted by permission from *Biochemical and Biophysical Research Communications.*)

Lipid peroxidation under *in vitro* conditions by liver microsomal and mitochondrial membranes (FIG. 12) was determined by measuring MDA production.[25] In the case of ad libitum fed rats, MDA production was higher in membranes from 24 month old rats than in those from 6 month old rats. Membranes from food restricted rats generated much less MDA than those from ad libitum fed rats. These *in vitro* findings are in agreement with the hydroperoxide content (TABLE 4)

TABLE 4. Hydroperoxide in Hepatic Membranes (nmoles/mg Protein)[a]

Age (Mos.)	Ad Libitum Fed		Food Restricted	
	Mitochondria	Microsomes	Mitochondria	Microsomes
6	1.31 ± 0.38	1.47 ± 0.33	0.44 ± 0.09	0.74 ± 0.12
12	1.57 ± 0.10	2.81 ± 0.15	0.99 ± 0.18	1.64 ± 0.17
24	1.98 ± 0.29	2.42 ± 0.29	1.24 ± 0.23	1.54 ± 0.10

[a] Data from Yu *et al.*[26]

of freshly isolated mitochondria and microsomes.[26] Several hepatic cytosolic antioxidants and related enzymatic activities (TABLE 5) were found to be influence by age and food restriction.[26] Glutathione reductase changed little with age but was maintained at significantly higher levels in food restricted than ad libitum fed rats. Reduced glutathione was stable through 18 months of age but fell by 24 months of age in ad libitum fed but not food restricted rats. Catalase activity declined slightly with age but food restriction maintained it at higher levels than in ad libitum fed rats. Mn-Superoxide dismutase and glutathione peroxidase changed little with age.

Food restriction was also found to influence the metabolism of malondialdehyde, a product of free radical damage.[26] The ability of hepatic mitochondria from ad libitum fed rats to oxidize malondialdehyde decreased with age and this decrease was partially prevented by food restriction (TABLE 6). This action may at least in part underlie the ability of food restriction to retard the age-associated accumulation of lipofuscin and related substances.

Yu *et al.*[27] recently postulated a cellular mechanism of action of food restriction based on the free radical theory of aging. In this postulation, aging is viewed to result from the continuous oxidative threat inherent from the basic metabolic processes of life; by protecting the cell from this threat, food restriction is felt to maintain the integrity of cellular structure and function even at advanced ages.

CONCLUSIONS

Two of the three questions addressed in the introductory paragraph appear to have been effectively addressed over the past 15 or so years. Are most age-associated processes influenced? The answer is yes. Most age-changes in physiological processes that have been studied are delayed or partially prevented by food restriction and almost all age-associated disease processes are retarded by food restriction. Does food restriction retard the primary aging processes? A

TABLE 5. Hepatic Cytosolic Antioxidants and Related Enzymes[a]

	6 Mos.		18 Mos.		24 Mos.	
	Gp. A	Gp. R	Gp. A	Gp. R	Gp. A	Gp. R
Superoxide dismutase	4.2 ± 0.3	4.8 ± 0.2	3.9 ± 0.1	4.9 ± 0.1	4.7 ± 0.4	4.8 ± 0.3
Catalase	529.4 ± 68.7	708.5 ± 158.6	590.0 ± 52.0	452.0 ± 127.2	288.9 ± 42.0	666.6 ± 88.1
GSH reductase	41.6 ± 2.8	49.8 ± 4.0	57.4 ± 2.0	67.5 ± 2.4	41.0 ± 2.7	52.3 ± 3.3
GSH transferase	527.4 ± 68.7	708.5 ± 158.6	590.0 ± 52.0	452.0 ± 127.2	473.6 ± 22.6	653.2 ± 40.6
GSH peroxidase	262.5 ± 14.9	279.6 ± 30.4	347.2 ± 217.1	249.1 ± 87.7	273.4 ± 26.4	292.6 ± 20.7
Glutathione	59.8 ± 7.7	50.9 ± 2.9	56.4 ± 13.1	51.5 ± 2.0	46.0 ± 5.0	62.3 ± 5.2
Ascorbic acid	2249.7 ± 221.3	2494.1 ± 266.2	2866.8 ± 273.5	2394.6 ± 401.3	1618.2 ± 94.6	1763.6 ± 99.0

[a] Gp. A refers to ad libitum fed and Gp. R to restricted groups. (Data in table are from Yu *et al.*[26])

TABLE 6. Hepatic Mitochondrial MDA Oxidation (nmol MDA Oxidized/mg Protein/10 Min)[a]

Age (Mos.)	Ad Libitum Fed Group	Restricted Group
6	1.47	1.64
12	1.12	1.33
18	1.01	1.12
22	0.69	—
24	—	1.14

[a] Data in table are from Yu *et al.*[26]

definitive answer must await the identification of the primary aging processes. However, the findings to date strongly indicate that a major site of action of food restriction is the primary aging processes. The salient findings supporting this view are the marked extension of the maximum life span and the breadth of the effects on age-changes in physiological processes and age-associated diseases. Such breadth must be the result of an action on a general fundamental process or processes rather than on a particular physiological event or a specific pathogenic process.

What are the mechanisms underlying the actions of food restriction on the aging processes? This question has proved to be most difficult to effectively explore. The major hypotheses proposed have been ruled out by recent studies. What has emerged from our studies is the concept that food restriction retards the aging processes by enabling the rodent to utilize fuel in less damaging ways than is the case for ad libitum fed rats. Our work has focused on the use of glucose and oxygen, both potentially toxic processes. Our findings indicate that glucose is used at sustained lower concentrations and therefore potentially less toxic levels in food restricted rats and that the generation of oxygen radicals is reduced and the mechanisms protecting the cell from their damaging action enhanced in food restricted rats. However, the exploration of this concept is in its infancy and further studies are needed to establish causality in the antiaging actions of food restriction.

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