TOXICOLOGIC PATHOLOGY ISSN:0192-6233

Volume 24, Number 6, 1996
Printed in U.S.A.

Possible Mechanisms Underlying the Antiaging Actions of Caloric Restriction*

EDWARD J. MASORO

University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7756

ABSTRACT

Restricting the food intake of mice and rats to well below that of *ad libitum*–fed animals markedly slows the aging processes. This action is reflected in an increase in longevity, a decrease in the age-associated increase in age-specific mortality rate, the maintenance of the physiological processes in a youthful state even at advanced ages, and the delaying of the onset or slowing of the progression or both of most age-associated diseases. The dietary factor responsible is the reduction in energy (caloric) intake. Many hypotheses have been proposed regarding mechanisms underlying this antiaging action. Hypotheses relating the antiaging action to the retardation of growth and development, the reduction of adipose mass, and the reduction of metabolic rate have been found to be wanting. Two of the proposed hypotheses have some evidence in their support. One involves the altered metabolic characteristics of glucose fuel use and of oxidative metabolism. The other relates to the enhanced ability of the rodents restricted in food intake to cope with challenges, which in turn has been linked to the glucocorticoid system and to the heat-shock protein system.

Keywords. Growth; body fat; metabolic rate; glucose–insulin system; oxidative damage; glucocorticoids; heat-shock proteins

INTRODUCTION

In 1935, McCay et al (26) reported the seminal finding that restricting food intake of rats starting from weaning and continuing for the rest of life markedly increased the longevity of the population. This finding has proven to be robust and highly reproducible, having been observed over the years in many different mouse and rat strains (35). The findings of studies carried out in our laboratory (38) with male Fischer-344 (F-344) rats that were either fed *ad libitum* or restricted to 60% of the *ad libitum* intake are a striking example. These findings are summarized by the survival curves in Fig. 1. Note the marked increase in the median and maximum length of life of the population that had a restricted food intake and how similar the survival curves were for the same dietary regimen in the 2 studies even though they were carried out 4 yr apart.

To make the text less cumbersome, the restriction of food will be referred to in the rest of this article as dietary restriction (DR) and, unless otherwise stated, the level of restriction used in the studies was 60% of the *ad libitum* intake. Although the effect of DR on the maximum length of life of the population strongly suggests that the aging processes have been retarded, many gerontologists feel that slowing the increase with age in the age-specific mortality rate would provide stronger evidence. Age-specific mortality rate refers to the fraction of the population entering an age interval that dies during that interval such as 31–32, 55–56, or 73–74 yr of age. In the case of most animal species, the age-specific mortality rate increases with increasing postmaturational age. This characteristic is often reported as the time it takes for the age-specific mortality rate to double—the mortality rate doubling time (MRDT), which in humans is about 8 yr. In 4 studies, each carried out at a different laboratory on different strains of male rats, the MRDT for the *ad libitum*–fed rats ranged from 99 to 104 days, with a mean value of 102 days, and for the rats undergoing DR it ranged from 187 to 210 days, with a mean value of 197 days (19). These findings provide powerful evidence that DR slows the aging processes.

DR maintains most but not all physiological processes in the youthful state even at advanced ages (20). The spectrum of processes so affected is great, ranging from female reproductive function to gene expression. In addition, DR delays the onset and/or slows the progression of almost all age-associated diseases including chronic nephropathy in rats and most tumors in mice and rats (20). DR clearly has the ability to globally retard the aging processes.

The question arose as to whether the antiaging action of DR was due to the reduction in intake of a specific nutrient and if so which one. This issue was intensively studied in our laboratory (18). The fact that in our work a semisynthetic diet was used facilitated the investigation. Two approaches were used. One involved feeding 2 groups of rats *ad libitum,* one group receiving the standard diet and the other the same diet except for the reduction in the amount of 1 component so as to reduce its intake to that of animals fed the DR regimen; for the findings to be interpretable, it was necessary for both groups to ingest a similar number of calories, which fortunately they did. The other approach was to restrict all dietary components by 40% except for 1. Based on the results of this research as well as the work of others, the conclusion is that it is the reduced intake of energy (calories) that underlies the antiaging action of DR.

* Address correspondence to: Dr. Edward J. Masoro, Department of Physiology, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7756.

0192-6233/96$3.00+$0.00

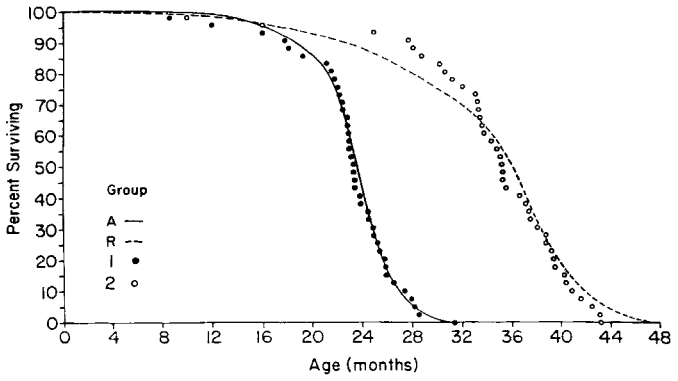

FIG. 1.—Survival curves for male F-344 rats fed *ad libitum* or restricted to 60% of the *ad libitum* intake. In the first study (39), started in 1975, survival curves were generated from 115 *ad libitum*–fed rats (Group A) and 115 dietary-restricted rats (Group R). In the second study (38), started in 1979, survival curves were generated from 40 *ad libitum*–fed rats (Group 1) and 40 dietary-restricted rats (Group 2). Although the small n of the second study did not yield the smooth curves of the larger n of the first study, results of the 2 studies are very similar. In both studies, DR markedly increased the median and maximum length of life. Reprinted from Yu et al (38) with permission.

GROWTH RETARDATION HYPOTHESIS

In their seminal 1935 paper, McCay et al (26) hypothesized that DR increases life span by slowing growth. In the ensuing years, this became a popular concept and led to the view, still held by some, that DR did not retard aging but rather extended the length of life by retarding growth and development. However, in 1982 Weindruch and Walford (34) found that DR started in mice at 12–14 mo of age (early middle age) caused a significant increase in the length of life although the magnitude of the effect was less than when DR was started at weaning. Subsequently, in a study carried out in our laboratory with male F-344 rats (38), it was found that DR started at 6 mo of age (young adults) was as effective in extending the age of the tenth percentile survivors and the maximum length of life as DR started at 6 wk of age. Moreover, DR started at 6 wk of age and stopped at 6 mo of age (i.e., during the rapid growth period of life) was not very effective in that it appeared that the extent of life extension related to the duration of the restriction and not to when in the life span it is employed. Thus, it must be concluded that the hypothesis of McCay and his associates is not valid.

BODY FAT HYPOTHESIS

In 1960, Berg and Simms (2) proposed that DR slows the aging processes by reducing the body fat content. This view was embraced by many because of the belief that in humans excess body fat is a risk factor for premature death. This hypothesis was evaluated in our laboratory in the male F-344 rat (4). DR was found to decrease the absolute fat mass and the percentage of body fat content. However, within the group of rats fed *ad libitum* there was no correlation between length of life and body fat, whereas within the DR group body fat and length of life were positively correlated. These findings certainly do not support the hypothesis of Berg and Simms nor does the study of Harrison et al (11) on *ob/ob* and C57BL/6J mice. The *ad libitum*–fed *ob/ob* mice were found to eat more food, to be fatter, and to live less long than the *ad libitum*–fed C57BL/6J mice. DR caused the *ob/ob* mice to live much longer than the *ad libitum*–fed C57BL/6J mice even though they were fatter. Indeed, they lived as long as the DR group of C57BL/6J mice, which were very lean. It seems clear that the reduction in body fat that occurs during DR does not play a causal role in its antiaging action.

METABOLIC RATE HYPOTHESIS

In 1977, Sacher (32) proposed that the antiaging action of DR is due to a decrease in metabolic rate per unit body mass. This hypothesis was widely accepted for several reasons. First, a relationship between the rate of aging and the metabolic rate has long been held, starting with studies by Rubner (30) early in this century. Second, in humans it has long been known that reducing food intake lowers the metabolic rate (9). Third, lowering the metabolic rate would be expected to decrease the generation of reactive oxygen compounds such as hydrogen peroxide and superoxide and hydroxyl radicals, which have been implicated in the aging processes (10). However, upon assessing the energy intake data from our studies with male F-344 rats, it was found that the energy intake per unit of lean body mass of rats fed *ad libitum* and of rats undergoing DR was similar for most of life (24). Further analysis revealed that the lean body mass was reduced proportionally to the reduction in caloric intake. Subsequently, McCarter and Palmer (25) found that oxygen consumption per unit of lean body mass is the same for most of the life span for *ad libitum*–fed male F-344 rats and those undergoing DR. These findings make clear that a reduction in metabolic rate need not occur for DR to have its antiaging action; that is, a level of DR, which markedly extended life span and increased the MRDT of male F-344 rats, did not decrease the metabolic rate.

METABOLIC CHARACTERISTICS OF FUEL USE HYPOTHESIS

Although a reduced metabolic rate appears not to be the factor responsible for the antiaging action, the nutritional studies strongly suggested that some aspect of fuel utilization is probably involved. This fact led us to propose that altered characteristics of fuel utilization are important in this action of DR. Indeed, studies carried out in my laboratory and in the laboratory of my colleague Dr. B. P. Yu have implicated 2 altered characteristics of fuel use as possible factors.

The glucose–insulin system seemed to be a likely possibility. It is known that chronic hyperglycemia and hyperinsulinemia in relatively young people cause damage such as microvascular disease, macrovascular disease, basement membrane thickening, impaired cellular immunity, cell cycle abnormalities, coronary artery disease, hypertension, and atherosclerosis (6, 8, 29, 33); moreover, similar problems are observed in people of ad-

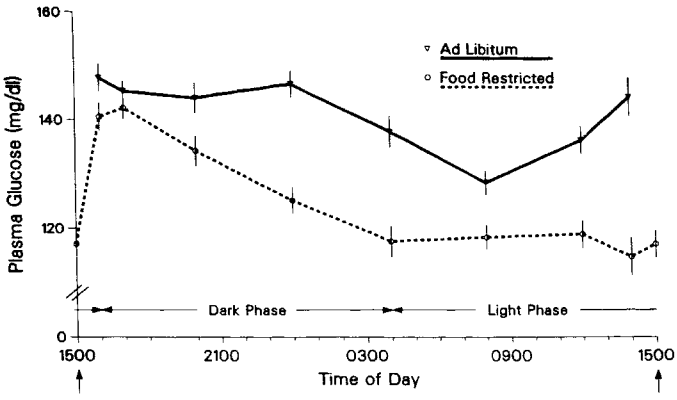

FIG. 2.—Diurnal pattern of plasma glucose concentrations in *ad libitum*–fed and dietary-restricted male F-344 rats. The points are means for 15 rats in the *ad libitum*–fed group and 15 rats in the DR group. The age range of the rats was 4–6 mo. The vertical arrow indicates the time of feeding the DR group. Reprinted from Masoro et al (22) with permission.

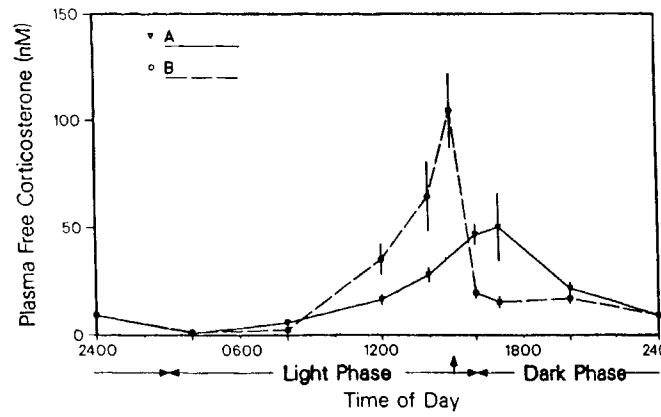

FIG. 3.—Circadian pattern of plasma-free corticosterone concentrations in the group fed *ad libitum* (Group A) and the DR group (Group B). The male F-344 rats were in the age range of 15–19 mo; the values are means for 21 rats in each group. Reprinted from Sabatino et al (31).

vanced age who for most of their life have had normoglycemia and normoinsulinemia. Thus, it seemed possible that normoglycemia and normoinsulinemia acting over a lifetime may at least in part underlie aspects of the aging phenotype. A life span longitudinal study was carried out in our laboratory on plasma levels of glucose and insulin in male F-344 rats fed *ad libitum* and in those undergoing DR. In Fig. 2, the diurnal pattern of plasma glucose concentration is shown for these 2 groups of rats during the age range of 4–6 mo (22). For most of the 24 hr, the rats in the DR group had plasma glucose levels significantly lower than those of the rats in the *ad libitum*–fed group. Similar findings were observed throughout the life span (23); the mean 24-hr levels of plasma glucose for the DR group ranged from 13 to 21 mg/dl below those of the DR group throughout the life span. Moreover, DR maintained plasma insulin levels markedly below those of the *ad libitum*–fed group throughout the life span, one-third to one-half the levels of the *ad libitum*–fed group. Surprisingly, the rate of glucose use as fuel over a 24-hr period was similar on a per-kilogram lean body mass basis for the 2 groups. DR must increase insulin sensitivity or glucose effectiveness as defined by Bergman (3) or have both actions. Importantly, by enabling effective use of glucose as fuel at lower lifetime plasma glucose and insulin levels, DR may reduce their long-term damaging actions, and this effect could well be an important component of the antiaging action of DR.

Another aspect of fuel use that is modified by DR is the damaging effects of the reactions involved in the use of oxygen (37). The metabolism of oxygen generates reactive oxygen-containing compounds (5), which, as already mentioned, have the capacity to damage and, thus, in the long-term, could well be the cause of much of the aging phenotype (14). Yu and his colleagues (15, 16, 36) have shown that DR attenuates the generation of reactive oxygen-containing compounds and increases antioxidant defense systems. These actions of DR could well be responsible for much of the antiaging action of DR.

GENERAL PROTECTIVE ACTION HYPOTHESIS

In addition to its antiaging action, DR enables rats and mice to better cope with the acute damaging action of a variety of stressors. In our laboratory, it was found that young as well as old male F-344 rats in the DR group lose a much lower percentage of body weight in response to the implantation of a jugular canula than rats in the *ad libitum*–fed group (21). Klebanov et al (13) reported that DR delays the occurrence and reduces the duration of the inflammatory response in the foot of young BALB/C mice due to the injection of carrageenan. Heydari et al (12) found that DR enables 20-mo-old male F-344 rats to survive an acute increase in environmental temperature much better than *ad libitum*–fed rats. Duffy et al (7) reported that DR protects rats from the toxic actions of drugs. It seems reasonable to suggest that these protective actions of DR allow rodents to cope successfully with stressors during periods of food shortage, thereby enabling them to reproduce when food again becomes available, and that the antiaging action of the sustained DR utilized in laboratory studies relates to this increased ability to cope with stressors, which in this case are the long-term, low-intensity damaging processes that cause aging.

Recent research may have uncovered the physiological vehicle or vehicles for this antiaging action. The adrenal glucocorticoids enhance the ability of mammals to successfully cope with a broad spectrum of stressors (27). In our studies with the male F-344 rat (31), it was found that in the DR group the afternoon peak levels of plasma-free corticosterone are elevated compared to the *ad libitum*–fed rat group (Fig. 3). In the case of the young animals, this increase is due to an increase in the level of plasma total corticosterone while with increasing age it is increasingly the result of decreasing plasma levels of corticosteroid-binding globulin that occurs in the DR but not in the *ad libitum*–fed group. It may be that rodents undergoing DR generate levels of plasma corticosterone

that are optimal for protecting them against acute stressors and the long-term aging processes. Certainly the findings of Pashko and Schwartz (28), on the role of the adrenal gland in the protection by DR of mice against the chemical induction of tumors, are in accord with this view.

The heat-shock protein system is another likely physiological vehicle underlying the increased ability to cope with stressors of animals undergoing DR. This system protects cells from many damaging agents in addition to heat (17). The ability to induce heat-shock protein, which decreases with advancing age, is enhanced by DR (1, 12). It is suggested that via the enhanced heat-shock protein system DR protects against aging due to agents directly damaging to cells and that via the enhanced adrenal cortical glucocorticoid system DR protects against aging due to excessive response of the sytemic defense systems such as the immune system and inflammatory processes.

REFERENCES

1. Aly KB, Pipkin JL, Hinson WG, Feuers RJ, Duffy PR, Lyn-Cook L, and Hart R (1994). Chronic caloric restriction induces stress proteins in the hypothalamus of rats. *Mech. Ageing Dev.* 76: 11–23.
2. Berg BN and Simms HS (1960). Nutrition and longevity in the rat. II. Longevity and onset of disease with different levels of intake. *J. Nutr.* 71: 255–263.
3. Bergman RN (1989). Toward physiological understanding of glucose tolerance, minimal model approach. *Diabetes* 38: 1512–1527.
4. Bertrand HA, Lynd FT, Masoro EJ, and Yu BP (1980). Changes in adipose mass and cellularity through the adult life of rats fed ad libitum or a life prolonging restricted diet. *J. Gerontol.* 42: 827–835.
5. Cardenas E (1989). Biochemistry of oxygen toxicity. *Annu. Rev. Biochem.* 58: 79–110.
6. Ducimetiere P, Eschwege E, Papoz JL, Richard JC, Claude JR, and Rosselin G (1980). Relationship of plasma insulin levels to the incidence of myocardial infarction and coronary heart disease mortality in middle aged population. *Diabetologia* 19: 205–210.
7. Duffy PH, Feuers RJ, Pipkin JL, Berg TF, Leakey JEA, Turturro A, and Hart RW (1995). The effect of dietary restriction and aging on the physiological response of rodents to drugs. In: *Dietary Restriction: Implications for the Design and Interpretation of Toxicity and Carcinogenicity Studies,* RW Hart, DA Neuman, and RT Robertson (eds). ISLI Press, Washington, D.C., pp. 125–140.
8. Ferrari P and Weidman P (1990). Insulin, insulin sensitivity and hypertension. *J. Hypertens.* 8: 491–566.
9. Garrow JS (1974). *Energy Balance and Obesity in Man.* Elsevier/North-Holland, Amsterdam.
10. Harman D (1981). The aging process. *Proc. Natl. Acad. Sci. USA* 78: 7124–7128.
11. Harrison DE, Archer JR, and Astole CM (1984). Effects of food restriction on aging: Separation of food intakes and adiposity. *Proc. Natl. Acad. Sci. USA* 81: 1835–1838.
12. Heydari AR, Wu B, Takahashi R, and Richardson AG (1993). The expression of heat shock protein 70 is altered by age and diet at the level of transcription. *Mol. Cell. Biol.* 13: 2909–2919.
13. Klebanov S, Shehab D, Stavinoha WB, Yongman S, and Nelson JF (1995). Hyperadrenocorticism attenuated inflammation, and the life prolonging action of food restriction in mice. *J. Gerontol.: Biol. Sci.* 50A: B78–B82.
14. Kristal BS and Yu BP (1994). Aging and its modulation by dietary restriction. In: *Modulation of Aging Processes by Dietary Restriction,* BP Yu (ed). CRC Press, Boca Raton, Florida, pp. 1–35.
15. Laganiere S and Yu BP (1989). Effect of chronic food restriction in aging rats: II. Liver cytosolic anti-oxidants and related enzymes. *Mech. Ageing Dev.* 48: 221–230.
16. Lee DW and Yu BP (1990). Modulation of free radicals and superoxide dismutases by age and dietary restriction. *Aging: Clin. Exp. Res.* 2: 357–362.
17. Lindquist S (1986). The heat shock response. *Annu. Rev. Biochem.* 55: 1151–1191.
18. Masoro EJ (1988). Food restriction in rodents: An evaluation of its role in the study of aging. *J. Gerontol.: Biol. Sci.* 43: B59–B64.
19. Masoro EJ (1992). The role of animal models in meeting the gerontologic challenge of the 21st century. *Gerontologist* 32: 627–633.
20. Masoro EJ (1993). Dietary restriction and aging. *J. Am. Geriatr. Soc.* 41: 994–999.
21. Masoro EJ (1994). Diet and the aging processes. *Clin. J. Sport Med.* 4: 262–267.
22. Masoro EJ, Katz MS, and McMahan CA (1989). Evidence for the glycation hypothesis of aging from the food-restricted rodent model. *J. Gerontol.: Biol. Sci.* 44: B20–B22.
23. Masoro EJ, Mc Carter RJ, Katz MS, and McMahan CA (1992). Dietary restriction alters characteristics of glucose fuel use. *J. Gerontol.: Biol. Sci.* 47: B202–B208.
24. Masoro EJ, Yu BP, and Bertrand HA (1982). Action of food restriction in delaying the aging process. *Proc. Natl. Acad. Sci. USA* 79: 4239–4241.
25. McCarter RJM and Palmer J (1992). Energy metabolism and aging: A lifelong study of Fischer 344 rats. *Am. J. Physiol.* 263: E448–E452.
26. McCay C, Crowell M, and Maynard L (1935). The effect of retarded growth upon the length of life and upon the ultimate size. *J. Nutr.* 10: 63–79.
27. Munck A, Guyre PM, and Holbrook NJ (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocrine Rev.* 5: 25–44.
28. Pashko LL and Schwartz AG (1992). Reversal of food restriction induced inhibition of mouse skin tumor promotion by adrenalectomy. *Carcinogenesis* 10: 1925–1928.
29. Rossetti L, Giaccari A, and De Fronzo RA (1990). Glucose toxicity. *Diabetes Care* 13: 610–630.
30. Rubner M (1908). *Das Problem der Lebensdauer und Seine Beziehungen Zum Wachstum und Ernahrung.* Oldenberg Munchen.
31. Sabatino F, Masoro EJ, McMahan CA, and Kuhn RW (1991). Assessment of the role of the glucocorticoid system in aging processes and in the action of food restriction. *J. Gerontol.: Biol. Sci.* 46: B171–B179.
32. Sacher GA (1977). Life table modifications and life prolongation. In: *Handbook of the Biology of Aging,* CE Finch and L Hayflick (eds). Van Nostrand Reinhold, New York, pp. 582–638.
33. Stout RW (1990). Insulin and atheroma. *Diabetes Care* 13: 631–654.
34. Weindruch R and Walford R (1982). Dietary restriction in mice beginning at 1 year of age: Effect on life span and spontaneous cancer incidence. *Science* 215: 1415–1418.
35. Weindruch R and Walford R (1988). *The Retardation of Aging and Disease by Dietary Restriction.* Charles C Thomas, Springfield, Illinois.
36. Yu BP (1993). Oxidative damage by free radicals and lipid peroxidation in aging. In: *Free Radicals in Aging,* BP Yu (ed). CRC Press, Boca Raton, Florida, pp. 57–88.
37. Yu BP (1995). Putative interventions. In: *Handbook of Physiology, Sect. 11: Aging,* EJ Masoro (ed). Oxford University Press, New York, pp. 613–631.
38. Yu BP, Masoro EJ, and McMahon CA (1985). Nutritional influences on aging of Fischer 344 fats. I, Physical, metabolic, and longevity characteristics. *J. Gerontol.* 40: 657–670.
39. Yu BP, Masoro EJ, Murata I, Lynd FT, and Bertrand HA (1982). Life span study of SPF Fischer 344 male rats fed ad libitum or restricted diets: Longevity, growth, lean body mass and disease. *J. Gerontol.* 37: 130–141.