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DIETARY RESTRICTION

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Abstract—Dietary restriction (DR) slows the rate of actuarial aging of rats and mice and in addition retards and/or delays many phenotypic characteristics of aging such as the age-associated deterioration of physiological systems and the occurrence and progression of age-associated disease. These antiaging actions result from a reduction of energy intake by the animal but are not due to a decrease in metabolic rate per unit of lean body mass. However, there is evidence that altered characteristics of fuel use (but not the intensity of fuel use) may underlie the antiaging action of DR. There is also evidence that DR has a general protective action in regard to damage caused by acute stressors; possibly a similar protective action in regard to damage caused by prolonged, low intensity aging processes plays a major role in the antiaging action of DR.

Key Words: Gompertz analysis, biological age, dietary restriction, energy intake, metabolic rate, carbohydrate metabolism, glycemia, insulinemia, glucocorticoids, heat shock proteins

INTRODUCTION

THE AGING phenotype is well known to all of us. It was clearly described around the turn of the seventeenth century by William Shakespeare in "As You Like It." In spite of this familiarity, so little is known about the basic biological nature of aging that a definition of aging acceptable to most biological gerontologists is difficult to achieve. The following is a working definition that is probably acceptable to most because controversial specifics are omitted: *deteriorative changes with time during postmaturational life that underlie an increasing vulnerability to challenges, thereby decreasing the ability of the organism to survive.* Although this is a definition only of the senescent period of life, in common usage aging refers to senescence rather than to all time-related events occurring during the life span. For this reason, in this article aging and senescence are used as synonyms.

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Assessment of aging at the population level

The rate of aging of a population is assessed by analyzing the mortality characteristics. This assessment involves measuring the age-specific mortality rate; i.e., the fraction of the population entering an age interval that dies during that interval (e.g., between 60 and 61 years of age). In most species living in environments that protect from premature death due to environmental hazards, there is an exponential increase in age-specific mortality rate with increasing postmaturational age (Finch, 1990). The slope of this exponential increase, often called the Gompertz Coefficient (G), is considered to be a quantitative index of the rate of aging. The Mortality Rate Doubling Time (MRDT) inversely relates to G (MRDT = $^{\ln 2}/_{G}$) and is often used instead of G because it is measured in the same units as life span (i.e., days, weeks, months, or years).

Recently, the use of the Gompertz analysis as an index of the rate of aging has been challenged because of the deviations from the monotonic exponential increase in age-specific mortality rate observed at advanced age in populations of some species (Carey *et al.*, 1992; Curtsinger *et al.*, 1992). In my opinion, these deviations do not negate the usefulness of Gompertz analysis in assessing the approximate rate of actuarial aging. The recent report of Brooks *et al.* (1994) supports this view.

Assessment of aging at the individual level

It is recognized that individuals within a population age at different rates, a fact that has led to the concept of biological age distinct from chronological age (Borkan and Norris, 1980). However, the means to measure the biological age of an individual has yet to be accomplished. Major efforts have been made to develop biomarkers that measure biological age (Baker and Sprott, 1991). However, the biomarkers or panels of biomarkers, that have been proposed, do not predict the remaining length of life as well as chronological age, a fact that casts doubt on their validity (Costa and McCrae, 1984). Thus, although physiological characteristics and disease processes in the individual are often assessed in relation to chronological age, the extent to which these assessments provide information on the biological age of the individual remains in doubt.

THE DIETARY RESTRICTION MODEL

Initial interest in dietary restriction (DR) emerged from studies showing that reduction in food intake below that of *ad libitum*-fed rats and mice increases the length of life. McCay *et al.* (1935) were the first to clearly show this effect, which has proven to be both robust and reproducible as is evident from the survival curves (Fig. 1) of male F344 rats from studies of Yu *et al.* (1982, 1985). In the two studies with male F344 rats, a 40% reduction in food intake resulted in about a 50% increase in median and maximum length of life and even though the two studies were carried out 4 years apart, the survival curves of each are nearly superimposeable.

Gompertz analysis

Assessment of the age-specific mortality rate by the Gompertz analysis shows that DR increases longevity because it slows the rate of actuarial aging (Sacher, 1977). The MRDT, assessed for four studies in which male rats of different strains were either *ad*

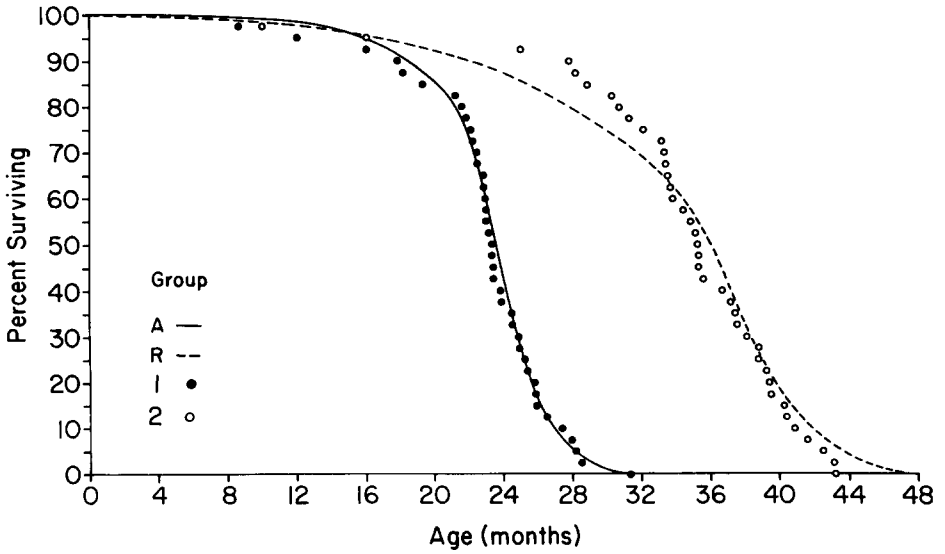

FIG. 1. Survival curves from two studies carried out 4 years apart on *ad libitum*-fed and dietary-restricted (60% of *ad libitum* intake starting at 6 weeks of age) male F344 rats. First study: Group A (n = 115), *ad libitum* fed; Group R (n = 115), dietary restricted. Second study: Group 1 (n = 40) *ad libitum* fed; Group 2 (n = 40) dietary restricted. Reproduced with permission from Yu *et al.* (1985).

libitum fed or restricted to about 60% of the *ad libitum* food intake, averaged 102 days (range, 99 to 104 days) for the *ad libitum*-fed rats and 197 days (range, 187 to 210 days) for the dietary-restricted rats (Masoro, 1992).

Aging phenotype

In addition to the slowing of actuarial aging, DR also influences most of the aging phenotypic characteristics. DR maintains most physiological processes in a youthful state even at advanced ages (Masoro, 1990). It also prevents or delays most age-associated disease processes; examples are nephropathy, cardiomyopathy, gastric ulcer, osteodystrophy, hypertension-related diseases, autoimmune diseases, cataracts, and a wide range of neoplastic diseases (Masoro, 1993).

Nutritional factors

The question arose as to which of the dietary components restricted in DR studies is responsible for the antiaging action. The use of semisynthetic diets assisted in answering this question. Two approaches were used: (1) the restriction of one component of the diet at a time, and (2) the restriction of all components but one. The findings from such studies show that the antiaging actions of DR are due to a reduction in energy intake and not to the reduced intake of a specific dietary component or dietary contaminant (Masoro, 1988).

MECHANISM OF ANTIAGING ACTION OF DR: DIRECT EFFECT

It was long believed that DR slows the aging processes by reducing the flux of one or more nutrients through the tissues of the organism. With the reduction in energy intake established as the responsible dietary factor, it was proposed that the antiaging action of DR is due to decrease in the intensity of metabolism, i.e., a reduction in specific metabolic rate (Sacher, 1977). This concept was challenged by the finding of Masoro *et al.* (1982) that energy intake per unit of body mass was somewhat greater in dietary restricted than in *ad libitum*-fed rats for most of the life span (Fig. 2). The reason for this surprising finding is that DR reduced the lean body mass proportionally to the reduction in energy intake (Yu *et al.*, 1982) but caused a disproportionately greater decrease in body fat mass (Bertrand *et al.*, 1980). The bottom line is that energy intake per unit of lean body mass is the same for *ad libitum*-fed rats and dietary-restricted rats over most of the life span.

The results of the study of oxygen consumption by McCarter and Palmer (1992) on *ad libitum*-fed and dietary-restricted male F344 rats while living in their home cages are in accord with the food intake findings. For most of the life span the dietary restricted rats had the same daily metabolic rate per unit of lean body mass as *ad libitum*-fed rats (Fig. 3). Clearly, the antiaging actions of DR cannot be due to a reduction in metabolic rate (i.e., the intensity of fuel use per unit of lean body mass).

The conclusion to be drawn is that a reduced flux of energy per unit of lean body mass is not responsible for the antiaging actions of DR. It is the reduced intake of energy per animal rather than per unit of lean body mass that causes the antiaging actions of DR.

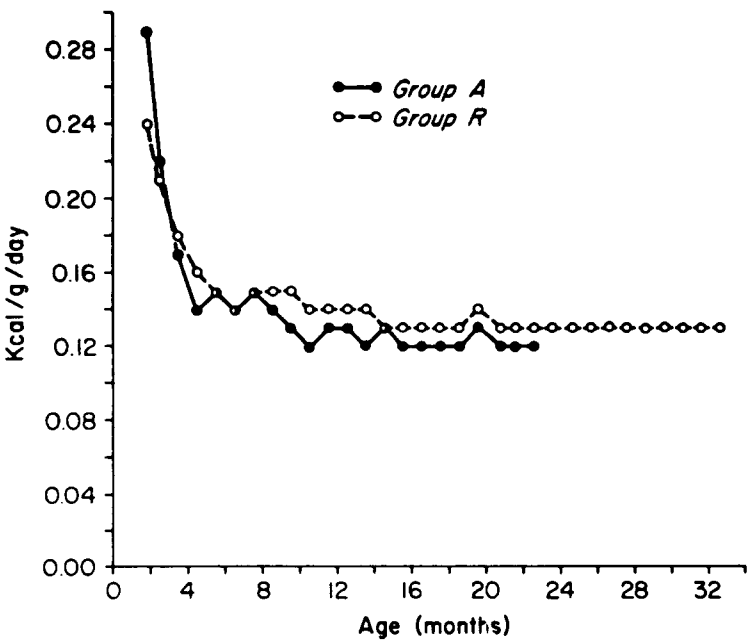

FIG. 2. Energy intake per gram body mass. The closed circles denote the *ad libitum*-fed rats (Group A) and the open circles the rats fed 60% of the *ad libitum* intake starting at 6 weeks of age (Group R). Reproduced with permission from Masoro (1985).

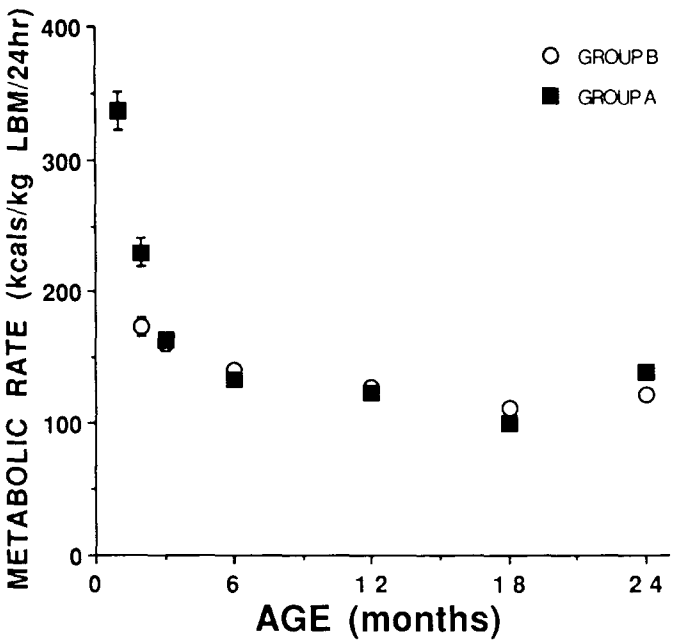

FIG. 3. Metabolic rate per unit of lean body mass per day for male F344 rats *ad libitum* fed (Group A) or restricted to 60% of the *ad libitum* intake starting at 6 weeks of age (Group B). Reproduced with permission from McCarter and Palmer (1992).

MECHANISM OF ANTIAGING ACTION OF DR: NERVOUS AND/OR ENDOCRINE SYSTEM AS MEDIATORS

How does a decreased intake of energy per animal, not reflected in a decreased energy flux per unit of "metabolic mass," retard the aging processes? The most likely explanation is that the nervous and/or endocrine system(s) act(s) as mediators that couple the reduced energy intake by the animal to aging processes in the tissues. Based on the currently available data base, two hypotheses have emerged in regard to this mediator action.

Metabolic characteristic of fuel use hypothesis

One of the hypotheses is that the reduction in energy intake brings into play endocrine and/or neural responses that alter the characteristics of fuel use. A study based on this hypothesis has revealed that the characteristics of carbohydrate fuel use are, indeed, altered by DR (Masoro *et al.*, 1992). In this life span longitudinal study, plasma glucose levels were found to be lower than those of *ad libitum*-fed rats throughout most of the day (Fig. 4). Through the lifespan, the mean 24-h plasma glucose concentration of the dietary restricted group ranged from 13 to 21 mg per dl below that of the *ad libitum*-fed group of the same age. Plasma insulin levels were markedly lower in dietary restricted rats throughout the life span, ranging from 22% to 63% of that of *ad libitum*-fed rats, depending on the time of day and the age of the rats. Surprisingly, the daily rate of carbohydrate fuel utilization per kg lean body mass was similar in dietary restricted

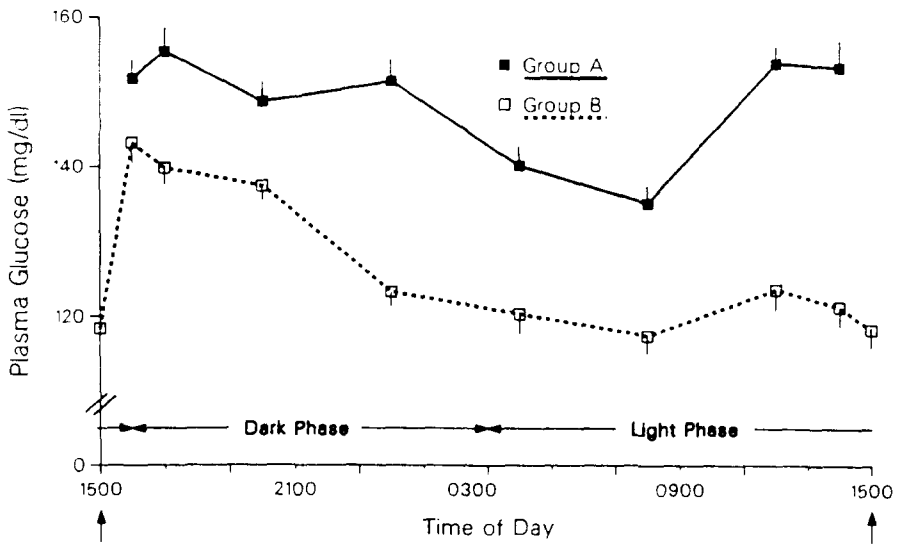

FIG. 4. Circadian pattern of plasma glucose concentration in male F344 rats during the age range of 9 to 13 months. Group A refers to *ad libitum*-fed rats and Group B to rats restricted to 60% of the *ad libitum* intake starting at 6 weeks of age. Short vertical arrows refer to time at which Group B received daily food allotment. Reproduced with permission from Masoro *et al.* (1992).

and *ad libitum*-fed rats. Either "glucose effectiveness" or "insulin sensitivity" or both, as defined by Bergman (1989), must be increased by DR.

This change in the characteristics of carbohydrate fuel use may, in part, underlie the antiaging action of DR. Hyperglycemia and hyperinsulinemia are known to be damaging (Reaven, 1989). If over a lifetime, normoglycemia and normoinsulinemia act as low intensity damaging agents (and there is no reason to believe a threshold concentration exists for their damaging action), then lower life span concentrations of plasma glucose and insulin should reduce the damage caused by these substances and thereby be at least part of the reason for the slowing of the rate of aging by DR.

The general protective action hypothesis

The other hypothesis is that DR puts the rodent into a protective mode in regard to all stressors (damaging agents) including those of the low intensity, long-term aging processes. Recent studies provide support for this view.

In our studies with male F344 rats, jugular cannula are often implanted. Following this surgical procedure, there is a loss of body weight. Two days after this surgical implantation, dietary restricted rats lose about one-third the amount of weight as do *ad libitum*-fed rats of the same age even when the data are normalized for initial body weight. Along the same line, Heydari *et al.* (1993) reported that DR increases the resistance of 20-month-old male F344 rats to hyperthermic stress. Also, Klebanov *et al.* (1993) found that DR attenuates the inflammatory reaction caused by the injection of carrageenan into the foot pad of male BALB/c mice.

The work of Sabatino *et al.* (1991) on the adrenal cortical system provides a possible

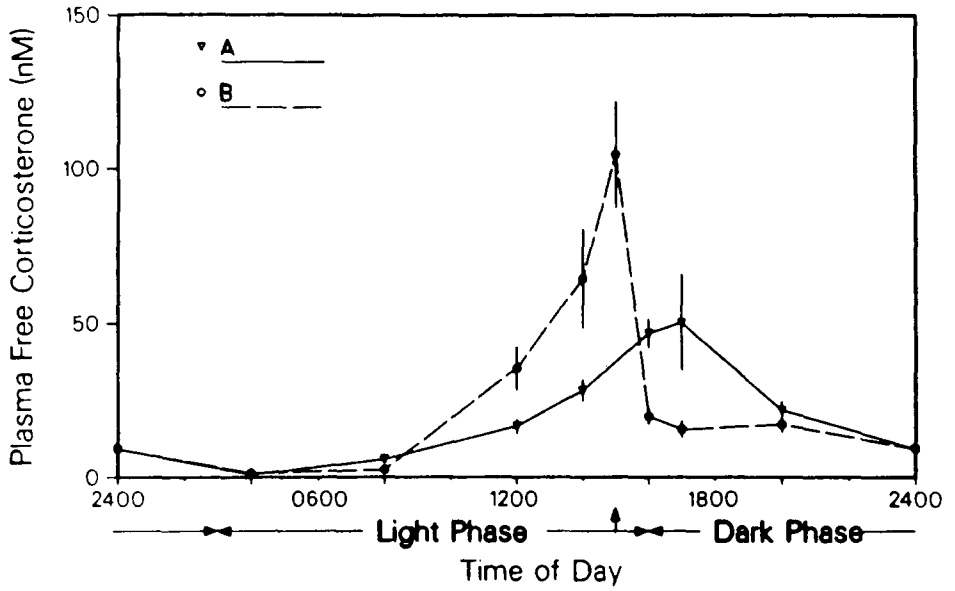

FIG. 5. Diurnal pattern of plasma-free corticosterone concentrations in *ad libitum*-fed rats (Group A) and rats restricted to 60% of the *ad libitum*-fed intake (Group B) in the age range of 15 to 19 months. Similar results were obtained in all age ranges. The rats were males of the F344 strain. Reproduced with permission from Sabatino *et al.* (1991).

basis for their resistance to stressors. This life span study of the diurnal pattern of the plasma-free corticosterone concentration revealed that DR causes elevated daily peak levels throughout the life span. The diurnal patterns obtained with 15- to 19-month-old rats are presented in Fig. 5. Glucocorticoids are known to be double-edged swords, too little resulting in inability to cope with stressors and too much causing damage (Munck *et al.*, 1984). Does DR result in optimally protective levels? If so, does this optimal level enable the rodent to resist the long-term, low intensity damaging action of aging processes? These questions require and deserve study.

Another protector from the adverse effects of a variety of stressors is the heat shock protein system (Lindquist, 1986). Recently, Heydari *et al.* (1993) reported that the ability of male F344 rats to express heat shock protein 70 (hsp 70) is enhanced by DR and that this enhanced ability is maintained into advanced ages. Thus, the heat shock protein system is a potential mechanism by which DR retards the aging processes. Whether and what role the nervous and/or endocrine system(s) play in the increased expression of heat shock protein and its enhanced expression in dietary restricted animals remains to be established and defined.

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