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Dietary Restriction: An Experimental Approach to the Study of the Biology of Aging

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I. Introduction

It is more than 60 years since McCay and his colleagues (McCay *et al.*, 1935) published their landmark paper showing that restricting food intake of rats soon after weaning increases the length of life. In the ensuing years, this finding has been confirmed repeatedly. In addition, the life-extending action has also been shown in mice and hamsters as well as in nonmammalian species, such as fish, flies, and water fleas. The life extension appears to be due to the slowing of the aging processes. Indeed, this phenomenon, which is often referred to as the antiaging action of dietary restriction (DR) or caloric restriction (CR), has been and is one of the most active areas of research in biological gerontology. Detailed coverage of the research on DR prior to 1993, including references to the original literature, can be found in a review article by Masoro (1993) and an encyclopedic book by Weindruch and Walford (1988). This chapter will focus on more recent findings; however, earlier work will be discussed as needed to provide a proper context.

II. Rodent Models

Studies on rats and mice, the animal models most used in DR research, have provided detailed information on DR's antiaging actions, as well as insights on possible underlying biological mechanisms. In these studies, the food intake of the DR group ranged from 50–70% of that of a control group; in most but not all studies, the control groups were fed *ad libitum*.

A. Antiaging Actions of Dietary Restriction

The mortality characteristics of populations of rats and mice undergoing DR have provided strong support for an antiaging action. The effects of DR on age changes in physiological processes and on the occurrence and progression of age-associated diseases have supplied additional support.

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1. *Mortality Characteristics*

As already stated, the findings of McCay *et al.* (1935) have been confirmed repeatedly in these numerous studies, both genders of several different genotypes of rats and mice have been used. In this regard, a study on the influence of DR on the survival characteristics of three rat genotypes and four mouse genotypes simultaneously maintained at the National Center for Toxicological Research in Jefferson, AR, is particularly impressive (Turturro *et al.*, 1999).

In addition, it has been found that life prolongation is robust even when DR is initiated in young adult life rather than soon after weaning (Yu *et al.*, 1985). Indeed, it has been found that DR initiated during early middle age significantly increases longevity in mice, though not as markedly as when started at earlier ages (Weindruch & Walford, 1982). This finding makes it clear that life prolongation by DR is not secondary to the prolongation of immaturity, a view initially proposed by McCay and his colleagues and long held by many. However, it is important to note that findings show that DR is not effective in prolonging life when initiated during late middle age or old age; when DR was started at 18 or 26 months of age in F344 × BN F1 hybrid male rats, there was no increase in longevity (Lipman *et al.*, 1998).

In addition to reducing the amount of food eaten, DR also changes the temporal pattern of food intake, with a meal-eating pattern replacing a nibbling pattern. This raises the possibility that the altered pattern of food intake rather than the reduced amount of food consumption is responsible for the antiaging action. This possibility was investigated, and the findings clearly show that reduced food intake is the factor responsible (Masoro *et al.*, 1995).

Gompertzian analyses of mortality characteristics of *ad libitum* fed and dietary restricted rat populations also strongly support the view that life prolongation by DR is due to its antiaging action. The analysis of mortality data from four rat studies, in which ingested food was about 60% of the *ad libitum* intake, showed that the mortality rate doubling time of the *ad libitum* fed rats ranged from 99–104 days and that of the dietary restricted rats ranged from 187–210 days (Holehan & Merry, 1986).

2. *Physiological Functions*

At advanced ages, most physiological processes of mice and rats on a DR regimen remain in a youthful state. Indeed, the number of functions thus affected by DR is so great that it is neither possible nor appropriate to provide anything approaching an encyclopedic coverage in a chapter of this length. Rather, our discussion will be limited to those effects that, in the author's opinion, may play an important part in the antiaging action.

DR modulates several fundamental cellular processes that may be intimately involved in aging. For example, it is quite likely that damage to DNA plays an important role in aging. In response to ultraviolet irradiation damage, DNA repair decreases with age in mouse splenocytes (Licastro *et al.*, 1988) and in rat liver and kidney cells (Weraarchakul *et al.*, 1989). It is significant that, in these studies, DR was found to retard the age-related decline in DNA repair activity. There is suggestive evidence that DR has a similar action in mouse skin cells (Lipman *et al.*, 1989). Also, cultured hepatocytes from old rats exhibit a compromised coupling of transcription and DNA repair, an age change that is prevented by DR (Guo *et al.*, 1998a,b). It appears that the effect of DR on DNA repair depends on the type of DNA damage (Haley-Zitlin & Richardson, 1993). Indeed, a study

indicates that the ability of DR to increase DNA repair is not universal; activities of DNA polymerases (enzymes involved in DNA repair) were found to be increased by DR in some, but not all, brain regions of the rat (Prapurna & Rao, 1996).

There is other evidence that DR helps maintain the stability of the nuclear and mitochondrial genomes with increasing age. DR markedly decreases the age-associated accumulation of mutations at the hypoxanthine phosphoribosyl transferase locus in mice (Dempsey *et al.*, 1993). Also, DR initiated in rats at middle age decreases the accumulation of skeletal muscle mitochondrial deletions and enzyme abnormalities and retards the loss of muscle fibers (Aspnes *et al.*, 1997).

Apoptosis eliminates damaged cells from the organism, and by so doing may protect the organism from deteriorative aspects of aging. Thus, it is significant that DR has been found to promote apoptosis in the liver of aging mice (Muskhelishvilli *et al.*, 1995) and the small intestine and colon of aging rats (Holt *et al.*, 1998). Moreover, apoptosis of preneoplastic cells is enhanced preferentially by DR in rats, thereby protecting them from carcinogenesis (Grasl-Kraup *et al.*, 1994). James and Muskhelishvili (1994) have also linked DR's increase in hepatic apoptosis in mice to a decreased incidence in hepatoma. On the other hand, DR prevents the age-associated increase in the susceptibility of rat hepatocytes to cell death induced by the administration of cycloheximide (Higami *et al.*, 1996).

Damaged proteins accumulate in cells with increasing age, which may well negatively impact cellular function. By degrading such proteins, proteolytic enzymes act to lessen this accumulation. However, protein degradation decreases with increasing age (Van Remmen *et al.*, 1995). DR attenuates the age-associated decrease in proteolysis (Ward, 1988), and this action may well be an important component of its antiaging action. It was suggested that DR's modulation of the age change in proteolysis might be due to alterations in proteosomes; however, studies designed to address this issue indicate that such is not the case (Shibatani *et al.*, 1996; Scrofano *et al.*, 1998).

The functioning of cells is regulated by hormones, cytokines, and neurotransmitters, which bind to cell receptors and alter cellular function by a complex pathway of interlinked chemical reactions collectively referred to as cellular signal transduction. Change with age in receptors and/or signal transduction could cause cellular dysfunction, and such changes do occur with increasing age. For example, cholinergic and dopaminergic stimulation of the formation of inositol phosphates (signal transduction pathway components) is decreased with increasing age in the brain of male F344 rats, and this decrease is prevented by DR (Undie & Friedman, 1993). Also, DR prevents the age-associated impairment in rat brain of the mitogen-activated protein kinase (MAPK) signal pathways (Zhen *et al.*, 1999).

With increasing age, there is a progressive impairment of the signal transduction pathway for growth hormone; DR was found to delay this impairment in mice (Xu & Sonntag, 1996a). Specifically, growth hormone activation of Stat–3 decreases with age, and DR prevents this decrease (Xu & Sonntag, 1996b). Is this action of DR important in its antiaging action? DR retards the age-associated decrease in protein biosynthesis (Van Remmen *et al.*, 1995). DR has been shown to increase the amplitude of growth hormone pulses in old animals (Sonntag *et al.*, 1995) and, by so doing, to sustain insulin-like growth factor-1 (IGF-1), which, in turn, sustains protein synthesis. By delaying age-associated impairment of the signal