Dietary Restriction and Aging

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The diet-restricted rodent model is widely used as a tool for the study of aging.[1] The aims of this review article are to evaluate this model and to assess the information about aging it has yielded as well as to project future directions of use.

ADVANTAGES AND DISADVANTAGES OF THE MODEL

The major advantage of this model is the robust evidence showing that dietary restriction retards the aging processes in laboratory rats and mice.[2] Indeed, the reproducibility of the findings in a variety of mouse and rat strains under widely different environmental circumstances is refreshing in the field of biological gerontology where repeatability is often a problem.

It was the effect of dietary restriction on longevity, first clearly shown by McCay and his associates[3] in the 1930's, that initially drew the attention of gerontologists. The survival curves in Figure 1 are from studies carried out in our laboratory with male F344 rats that were either fed *ad libitum* or restricted to 60% of the food intake of the *ad libitum* fed rats.[4,5] The effects on longevity and the reproducibility are typical for studies of dietary restriction. The fact that dietary restriction markedly increases the maximum life span was felt to provide strong evidence in support of its retarding the aging processes. Finch et al[6] have recently challenged the concept that maximum life span can be used as a reliable index of the rate of aging. They provide reason to believe the Gompertz mortality rate coefficient (G) is a better guide to the rate of aging; ie, if a manipulation slows the rate of aging of a population it should decrease G. The Mortality Rate Doubling Time (MRDT) relates to G as follows:

$$\text{MRDT} = \frac{\ln 2}{G}$$

Therefore, if dietary restriction retards the aging processes, it should increase the MRDT. In four studies using different strains of rats[7] in which similar levels of dietary restriction were employed, the MRDT ranged from 99 to 104 days for populations of *ad libitum* fed rats and 187 to 210 days for populations of diet-restricted rats. This effect on MRDT provides strong evidence for a retardation of the aging processes by dietary restriction.

Dietary restriction also maintains a broad array of physiological processes in a youthful state. The entries in Table 1 make evident the breadth of this action, but in no way do they represent all such actions that have been documented, let alone those processes yet to be studied.

Even more striking than the ability to modulate age-changes in physiological processes are its effects on age-associated disease processes in many different rat and mouse strains. Many such actions are presented in Table 2.

Although the strong evidence for its ability to retard aging processes is the major reason for the wide use of diet-restricted rodents for aging research, the very breadth of these actions presents disadvantages. Indeed, so broad are its anti-aging effects that dietary restriction provides little more in the way of guidance for the direction of aging research than do the phenotypic manifestations of aging. Indeed, the view of most investigators is that only by learning the mechanisms by which dietary restriction retards aging processes will the use of this model provide the information needed to gain a basic understanding of aging processes or the data base required for the development of practical interventions of human aging.

DIETARY RESTRICTION AND PRIMARY AGING PROCESSES

A premise long held by most biological gerontologists is that there are primary aging processes.[40] Indeed, much of the aging phenotype is believed to be secondary or further removed expressions of the primary aging processes. For a long time, it was felt that a single primary aging process was responsible for most of the aging phenotype. However, most contemporary biological gerontologists feel several such processes exist and that they differ among species and, possibly, within species.

A logical extension of this view is that dietary restriction retards one or more of these primary processes. Indeed, the scope of the anti-aging actions of dietary restriction is so great that the processes affected must be those of major importance.

Studies on the nature of the dietary factor responsible for the antiaging action have provided a clue to the possible nature of the primary processes that are retarded. It is the restriction of energy intake and not of a specific nutrient that underlies the anti-aging action of dietary restriction.[41,42]

This finding suggests the obvious possibility that dietary restriction retards the aging processes by reducing the metabolic rate. Indeed, there is an old and large literature suggesting that the rate of energy metabolism

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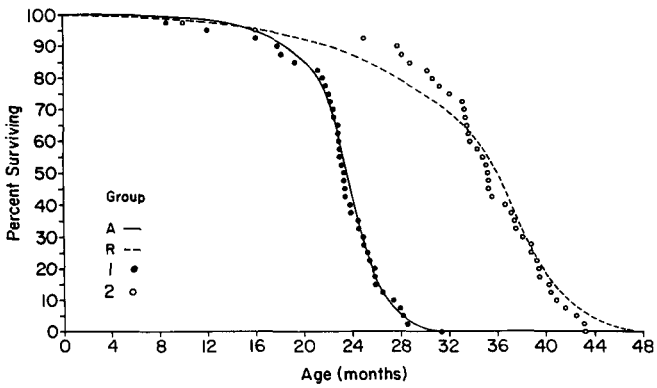

FIGURE 1. Survival curves from two studies carried out in our laboratory on *ad libitum*-fed and diet-restricted (60% of *ad libitum* fed intake) male F344 rats.[4,5] Group A (n = 115) and Group R (n = 115) rats, *ad libitum* fed and diet-restricted, respectively, are the animals used in the first study.[4] Group 1 (n = 40) and Group 2 (n = 40) rats, *ad libitum* fed and diet-restricted, respectively, are the animals used in the second study.[5] The two studies were carried out 4 years apart. The small number of the second study did not yield the smooth curves of the larger number of the first study. Nevertheless, the respective curves of the two studies are remarkably similar. Reproduced with permission from Yu et al.[5]

TABLE 1. PHYSIOLOGICAL PROCESSES MAINTAINED IN YOUTHFUL STATE BY DIETARY RESTRICTION OF RATS AND/OR MICE

Physiological Process	Literature Reference
Plasma cholesterol level	Masoro et al[8]
Parathyroid hormone level	Kalu et al[9]
Plasma calcitonin level	Kalu et al[10]
Response of adipocytes to lipolytic hormones	Bertrand et al[11]
Dopamine receptors in corpus striatum	Roth et al[12]
Ability to learn a maze	Ingram et al[13]
Locomotor activity	Yu et al[5]
Vascular smooth muscle tension development	Herlihy and Yu[14]
Gamma crystallin level in lens	Leveille et al[15]
Female reproductive function	Merry and Holehan[16]
Immune function	Fernandes[17]
Collagen cross-linking	Harrison and Archer[18]
Hepatic protein biosynthesis	Ward[19]
Hepatic proteolysis	Ward[20]
DNA repair in splenocytes	Licastro et al[21]
α-2u-globulin expression	Chatterjee et al[22]
Blood glutathione level	Lang et al[23]
Hepatic cytochrome oxidase content	Rumsey et al[24]

is a major factor underlying aging. However, studies carried out in our laboratory have shown that dietary restriction can retard the aging processes without reducing the metabolic rate, ie, the rate of energy metabolism per unit of "metabolic mass."[43]

Although a reduction in energy intake or energy expenditure per unit of "metabolic mass" is not responsible for the antiaging actions of dietary restriction, a decrease in energy intake per animal is certainly involved. Since a decrease in intensity of energy metabolism and/or flux of energy nutrient per unit of tissue mass is not the signal, the challenge that emerges is that of learning how a reduction in energy intake per animal is coupled to the aging processes. Based on currently available data, it seems likely that neural and/or endocrine regulatory systems are involved. Our working hypothesis is that the characteristics, but not the rate, of energy metabolism are modulated by dietary restriction because of alterations in endocrine or neural regulatory systems. It is further postulated that it is this modulation in characteristics of fuel use that underlies the antiaging actions of dietary restriction.

Our research on carbohydrate metabolism has yielded findings consistent with this working hypothesis. A longitudinal study of the diurnal pattern of plasma glucose concentration was executed.[44] Through most of the day, diet-restricted rats maintained plasma

TABLE 2. AGE-ASSOCIATED PATHOLOGIC LESIONS RETARDED BY DR

Pathologic Lesion	Species	Strain	Sex	Reference
Nephropathy	Rat	F344	M	Maeda et al[25]
Nephropathy	Rat	Sprague-Dawley	M & F	Berg and Simms[26]
Nephropathy	Rat	Osborne-Mendel	M	Saxton and Kimball[27]
Nephropathy	Rat	Wistar	M	Tucket et al[28]
Cardiomyopathy	Rat	F344	M	Maeda et al[25]
Cardiomyopathy	Rat	Sprague-Dawley	M & F	Berg and Simms[26]
Gastric Ulcer	Rat	F344	M	Maeda et al[25]
Osteodystrophy	Rat	F344	M	Shimokawa et al[29]
Hypertension-related lesions	Rat	SHR	M	Lloyd[30]
Autoimmune kidney disease	Mouse	(NZBXNZW)F_1	M & F	Fernandes et al[31]
Cataract	Mouse	Emory	M & F	Taylor et al[32]
Lymphoproliferative disease	Mouse	MRL/mp-lpr/lpr	M	Kubo et al[33]
Mammary tumors	Mouse	DBA	F	Tannenbaum[34]
Lung tumors	Mouse	ABC	F & M	Tannenbaum[34]
Leukemia	Mouse	Ak	F & M	Saxton et al[35]
Leukemia	Rat	F344	M	Shimokawa et al[36]
Lymphoma	Mouse	C3B10RF_1	F	Weindruch et al[37]
Pituitary adenoma	Mouse	HaniNMRI	F	Rehm et al[38]
Pancreatic adenoma	Rat	Sprague-Dawley	M	Ross and Bras[39]
Pituitary adenoma	Rat	Sprague-Dawley	M	Ross and Bras[39]
Pituitary adenoma	Rat	F344	M	Shimokawa et al[36]

glucose levels below those of *ad libitum* fed rats. However, for about 3 hours after receipt of the daily food allotment, the diet-restricted rats had plasma glucose levels that approached those of the *ad libitum* fed rats. This basic pattern was sustained with only minor variation throughout the life span. The mean 24-hour plasma glucose concentration was calculated from the diurnal pattern of plasma glucose concentration and is presented in Table 3. The diet-restricted rats maintained mean 24-hour plasma glucose concentrations significantly below those of *ad libitum* fed rats, ranging from 13 to 21 mg/dL lower, depending on the age range.

Plasma insulin levels were measured 4 hours after the end of the dark phase of the light cycle (when plasma glucose levels are lowest for both *ad libitum* fed and diet-restricted rats) and 1 hour after the start of the dark phase when plasma glucose levels were highest in both dietary groups. At both times, plasma insulin levels were markedly lower in diet-restricted than in *ad libitum* fed rats, ranging from 22% to 63% of the concentration in the *ad libitum* fed rats, depending on time of sampling and age of the rat.[44]

The plasma glucose and insulin concentrations, the rate of oxygen consumption, and the respiratory quotient were measured in the same rats over a 5-day period.[44] Based on these measurements, it was concluded that diet-restricted rats and *ad libitum* fed rats have a similar daily rate of use of glucose fuel per $Kg^{3/4}$ body weight and that dietary restricted rats are able to use glucose fuel at this rate while maintaining lower plasma glucose concentrations and markedly lower plasma insulin concentrations. Clearly, either "glucose effectiveness," "insulin sensitivity," or both must be increased by dietary restriction.

Hyperglycemia and hyperinsulinemia are known to have damaging actions. Therefore, it is conceivable that a lifetime of normoglycemia and normoinsulinemia may be damaging and, in this way, contribute in a major way to senescence. If so, much of the antiaging action of dietary restriction might well be due to its ability to maintain lower life span concentrations of plasma glucose and insulin without interfering with the use of glucose as fuel. Although our findings are consistent with this view, they in no way establish its validity. The influence of dietary restriction on carbohydrate metabolism, plasma glucose concentration, and plasma insulin concentration may merely be another of its many actions with no causal role in the antiaging action.

TABLE 3. Mean 24-Hour Plasma Glucose Concentration*

Age Months	Control		Diet-Restricted	
	n	mg/dL	*n*	mg/dL
3–7	21	136 ± 2**	21	119 ± 2
9–13	21	147 ± 2	21	126 ± 2
15–19	21	149 ± 2	21	131 ± 2
21–25	15	148 ± 3	19	135 ± 3
27–31			13	127 ± 3

* *Reproduced with permission from Masoro et al*[44].
** *Mean ± SE.*

The influence of dietary restriction on oxidative damage could be another vehicle that alters the characteristics of fuel utilization in a way that retards aging processes. Much evidence has been gathered that indicates that diet-restricted rodents suffer less oxidative damage than *ad libitum* fed rats even though the rate of oxygen consumption per unit of lean body mass is not decreased.[45] Diet-restricted rodents accumulate less lipofuscin and undergo less lipid peroxidation with age than *ad libitum* fed rats. Also, systems that protect against such damage such as glutathione, glutathione reductase activity, and catalase activity are upregulated by dietary restriction. These findings are consistent with the Free Radical Theory of Aging and with the antiaging action of dietary restriction resulting from the ability of this dietary manipulation to decrease free radical damage. Although these findings are provocative, they do not establish that protection from oxidative damage plays a causal role in the antiaging actions of dietary restriction.

DIETARY RESTRICTION AND GENERAL PROTECTIVE PROCESSES

The view has been proposed recently that the specific processes underlying senescent deterioration may be many and that they differ among species as well as within species.[46] If so, the broad spectrum of antiaging actions shown for dietary restriction becomes difficult to explain on the basis of it acting directly on primary aging processes to retard them. It seems unlikely that this dietary manipulation can directly interact with many different primary processes.

Is there a mode of action by which dietary restriction may have its many antiaging actions other than by directly influencing primary aging processes? One possibility is that it protects the animal from a wide array of harmful events including the aging processes. Support for such a view has emerged from two lines of study.

One line focuses on adrenal cortical function. Sabatino et al[47] carried out a longitudinal study on the diurnal pattern of plasma-free corticosterone concentration in *ad libitum* fed and diet-restricted male F344 rats. The plasma concentration of free corticosterone is low between 8 pm and 8 am, with relatively high levels occurring from 8 am to 8 pm in both diet-restricted and *ad libitum* fed rats. The circadian pattern for the 21 to 25 month old rats is shown in Figure 2; qualitatively similar patterns were observed throughout the life span. The important point is that throughout the life span, plasma-free corticosterone concentration reaches much higher levels each afternoon in diet-restricted rats than in *ad libitum* fed animals.

The question arises as to the role, if any, of elevated plasma-free corticosterone concentrations in the antiaging action of dietary restriction. It is known that glucocorticoids play an important protective function in the response of animals to challenges from a broad array of stressors.[48] Do the elevated plasma-free corticosterone levels in diet-restricted animals provide a broad protection against damage, including the damaging actions of aging processes? Unpublished data

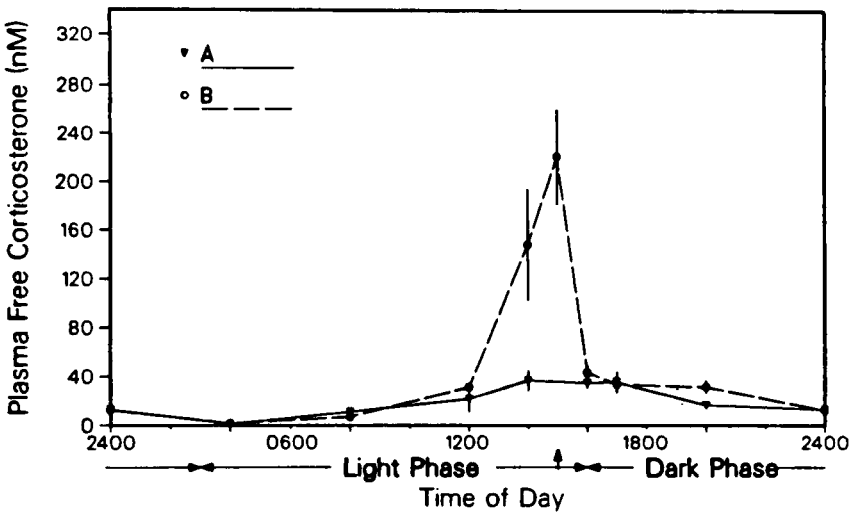

FIGURE 2. Circadian pattern of plasma free corticosterone in male F344 rats in the age range of 21 to 25 months. Group A denotes *ad libitum* fed rats and Group B diet-restricted rats fed 60% of the *ad libitum* fed intake. Reproduced with permission from Sabatino et al.[47]

from studies in our laboratory on the response of diet-restricted and *ad libitum* fed male F344 rats to the surgical implantation of a jugular canula indicate such may be the case. Diet-restricted rats lost a much lower percentage of body weight during the 48 hours following this surgical procedure than did *ad libitum* fed rats. Unpublished findings of Simon Klebanov and James Nelson on foot pad edema in male F344 rats induced by injection of carregeenon also support this view. The edema was delayed and of shorter duration in diet-restricted compared with *ad libitum* fed animals. It appears that diet-restricted rats met these acute challenges more effectively than *ad libitum* fed animals. Of course, these studies do not establish the elevated plasma free corticosterone levels as the responsible factor. Also, such studies do not provide information on the role of elevated plasma free corticosterone levels as protectors against the damaging actions of aging processes. Nevertheless, the information to date is sufficiently provocative to warrant further studies aimed at investigating the role of the daily elevation of plasma-free corticosterone concentration in the antiaging action of dietary restriction.

The other line of study focuses on heat shock proteins, also known as stress-induced proteins. It is believed that this class of proteins protects animals against the adverse effects of various stressors. Heydari et al[49] have found that the ability of hepatocytes to express heat shock protein 70 (hsp 70) after a mild heat stress is decreased with increasing age. This decrease in hsp 70 expression is due to a decline in hsp 70 transcription. Dietary restriction prevents this age-associated decline in hsp 70 expression. At least in part, this action of dietary restriction relates to its ability to maintain an active heat shock transcription factor. This ability may be another general protective mechanism underlying the antiaging actions of dietary restriction.

PROBLEM OF ESTABLISHING CAUSALITY

Although many of the actions of dietary restriction have been suggested to play a causal role in the antiaging action, clear evidence for causality has not been obtained for any of them. This is a generic problem for aging research that probably relates to the fact that multiple interacting processes occurring over an extended period of time are likely to be involved in aging. If so, designing studies to establish cause and effect would be expected to be a formidable task.

Related to this problem are the difficulties that have been encountered in studies aimed at determining if dietary restriction has antiaging actions in mammalian species other than rodents, particularly in non-human primates and humans. Because of the long life span of these species, it is difficult, if not impossible, to determine effects of this dietary manipulation on longevity or on Gompertzian aging. Therefore, investigators have tried to use physiological biomarkers to assess the antiaging action of dietary restriction in these long-lived species. The information published to date on non-human primates has not been impressive but, in fairness, these studies are just beginning.[50,51] However, even if impressive findings on putative biomarkers ultimately emerge, the recent paper of Walford et al[52] on humans makes evident the difficulties in the interpretation of the findings in regard to evidence for an antiaging effect of dietary restriction in long-lived species. In that human study, the eight subjects in the Biosphere 2 project had been on a low energy intake (1780 Kcal per day) for 6 months. The authors point out that the physiological changes in these people attributable to dietary restriction are similar to those observed in diet-restricted rodents. For example, the reduction in plasma glucose observed in rats was found in these subjects. However, as discussed above, there is not yet evidence that the sustained low levels of plasma glucose in rats are causally related to the life-extending or other antiaging effects of dietary restriction in this species. Thus, the biomarkers measured by Walford et al do not provide clear evidence for an antiaging action of dietary restriction in humans, and it is anticipated that similar interpretational problems will be encountered in the non-human primate studies.

Experimental designs must be developed that can causally link biomarkers to the antiaging action of dietary restriction. Practically, the first stage in developing such designs must make use of short-lived ani-

mals such as laboratory rodents. Only if the first stage is successful should the challenge of developing designs for extending the research to long-lived mammals be undertaken.

In regard to the first stage, transgenic rodent models may provide an approach. For example, the issue of the causal role of low plasma glucose and insulin levels may be experimentally approached using transgenic mice in which the level of glucose transporter molecules have been appropriately manipulated.

SUMMARY AND CONCLUSIONS

The diet-restricted rodent model has been and is a major tool in experimental biogerontology. A spectrum of findings indicates that dietary restriction retards the aging processes of mice and rats, the most salient of which is the increase in mortality rate doubling time. It also maintains many physiological processes in a youthful state and, most strikingly, retards or prevents almost all age-associated disease processes.

Current emphasis is on the mechanisms underlying the anti-aging actions of dietary restriction. The major effort for determining mechanism has focussed on putative primary aging processes. A clue has emerged from the findings that it is the restriction of energy intake that is the dietary factor responsible for the anti-aging actions. However, reducing the metabolic rate is not involved. The challenge is to learn how the reduction of energy intake per animal (not per unit of body mass) is coupled to the retardation of aging processes.

One of our working hypotheses is that dietary restriction alters nervous and/or endocrine functions that influence the characteristics (not the rate) of fuel use; this modulation in fuel-use characteristics is proposed to retard the aging processes. Our findings on carbohydrate metabolism are in accord with this view. Diet-restricted rats can use carbohydrate fuel as effectively as *ad libitum* fed rats while maintaining lower plasma glucose and insulin level. Maintenance of these low levels may protect against long-term damaging actions of these substances. Dietary restriction also protects against oxidative damage and, of course, oxidative damage is probably an inevitable component of fuel use. This specific protective effect is another way dietary restriction may interact with a primary aging process. The involvement of neural or endocrine mechanisms in this protection remains to be defined.

It is possible that dietary restriction generates a protective action against damage, including that due to aging, rather than acting directly on specific aging processes. The ability of dietary restriction to maintain a life span elevation of plasma glucocorticoid levels may provide one such protective action. Another potentially protective action is the ability of dietary restriction to prevent the age-related loss in the expression of stress-induced heat shock proteins; these proteins are known to protect against many damaging agents, and aging processes may well be included.

Many of the effects of dietary restriction have been proposed to be causally involved in its antiaging actions, but for none is the supporting evidence adequate. This lack of information about causality has made it impossible to use biomarkers to ascertain if dietary restriction is retarding the aging processes of humans and non-human primates. Future research should be aimed at the development of experimental designs that will yield information on the causal relationship between biomarkers and the antiaging action of dietary restriction.

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