Age, Vol. 1, Pages 130-142, October, 1978

DIET AND LIFE EXTENSION IN ANIMAL MODEL SYSTEMS *

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Abstract

Recent studies have shown that beneficial effects can be brought about when underfeeding is initiated in adult as well as young growing animals. In addition, such dietary manipulations have been shown to delay the onset of a variety of diseases although its relationship to total incidence has not been established. It has been proposed that dietary restriction reduces protein synthesis and increases lifespan by retarding genetic informational transfer during early life and reducing the use of the genetic code and thereby minimizing genetic imperfections as they may occur during late life.

Introduction

Dietary restriction has been shown to increase the lifespan of laboratory animals. In general, dietary restriction has been brought about by: 1) reducing the daily intake of a nutritionally adequate diet (one which supports maximal growth); 2) intermittently feeding a nutritionally adequate diet (e.g., feeding every second, third, or fourth day); and 3) feeding *ad libitum* a diet containing insufficient amounts of protein to support maximal growth.

Any increase in lifespan associated with dietary manipulations is generally believed to be due to a restriction of dietary calories. However, most studies in an attempt to accomplish caloric restriction have restricted the intake of a nutritionally adequate diet so that not only has the caloric intake been reduced but also the protein and other dietary components as well. It must be recognized that it is experimentally difficult to hold all dietary components constant and reduce only calories. In order to achieve only caloric restriction under *ad libitum* conditions, there must be adjustments in the diets according to an animal's intake which changes markedly with growth and is dependent upon dietary composition. This has been accomplished (1) and the data indicated that restriction of calories indeed increased the life span of C_3 H mice.

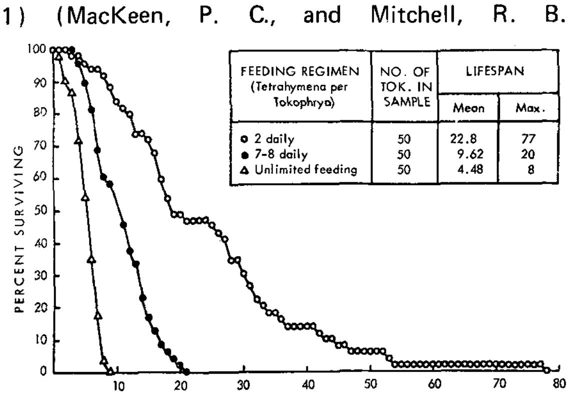
* This is the first paper of the mini-symposium on nutrition and aging organized by Charles H. Barrows, Jr., and presented on Thursday, September 29, 1977 as part of the 7th Annual Meeting of the American Aging Association in New York City.

The *ad libitum* feeding of a diet containing insufficient amounts of protein to support maximal growth has been shown to increase the life span of both young growing and adult animals (2). However, it is not clear the degree to which caloric restriction occurs under these experimental conditions. For example, it has been reported that reducing dietary protein did not affect the caloric intake of adult rats (3). However, Ross (4) reported that the caloric intake of rats fed a synthetic diet containing 8% casein was reduced when compared to that of animals fed a commercial diet. Similar data has been reported by Barrows, et al. (5). In contrast, Stoltzner (6) has reported a marked increase in the caloric intake of BALB/c mice fed *ad libitum* diets containing low amounts of protein. Therefore, on the basis of data presently available, it is not possible to conclude that calories are the sole dietary component which influence life span.

It has been generally believed that nutritional manipulations which increase lifespan had to be imposed during early growth. This concept originated as a result of the early work of Minot (7,8) postulating that senescence follows the cessation of growth. In addition, McCay, et al. (9,10), showed that increased lifespan of rats was associated with growth retardation. Furthermore, Lansing (11) indicated that aging in the rotifer involves a cytoplasmic factor the appearance of which coincides with the cessation of growth. However, more recently, studies have indicated that dietary restriction imposed in adult life was effective in increasing lifespan. Therefore, the results of experiments reported here have been divided wherever possible into whether dietary restriction was imposed on young growing animals or on adult organisms.

Life Span

Young Growing Animals. Increased lifespan associated with underfeeding has been reported in the following animal model systems: *Tokophyra* (Figure 1) (MacKeen, P. C., and Mitchell, R. B.:



FEEDING REGIMEN (Tetrahymena per Tokophrya)	NO. OF TOK. IN SAMPLE	LIFESPAN	
		Mean	Max.
o 2 daily	50	22.8	77
• 7-8 daily	50	9.62	20
Δ Unlimited feeding	50	4.48	8

Figure 1. The Percent Survivorship of *Tokophrya lemnarum.*

Cytophotometric determination of cytoplasmic azure B RNA levels throughout the lifespan of *Tokophrya Lemnarum.* The Gerontologist, 15: No. 5, 27, 1975); *Campanularia flexuosa* (Figure 2) (Brock, M. A.: Gerontology Research Center, National Institute on Aging, Baltimore, Maryland; personal communication); *Daphnia* (Figure 3) (12); rotifers (Table 1) (13); *Drosophila* (14); and fish (Figure 4) (15). In addition, a number of laboratory experiments have been carried out on rodents.

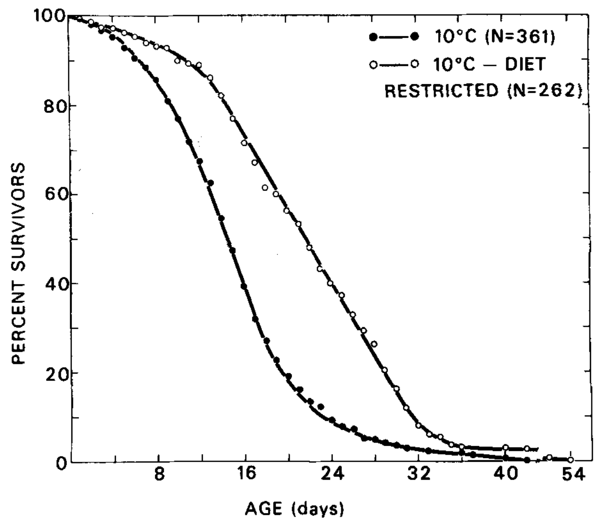


Figure 2. The Percent Survivorship of *Campanularia flexuosa* Fed Artemia Daily or Every Third Day.

Table 1. Effect of Nutrition on Life Span of Rotifers

		Mean life span (days)		
		Diet [a]		
		I	II	III
Exp. 1	Mn	35.7	43.8	58.6
	omn	±2.1	±3.0	±1.9
Exp. 2	Mn	36.0	45.6	56.5
	omn	±1.2	±2.5	±2.2
Exp. 3	Mn	29.0	46.2	49.1
		±2.8	±3.2	±2.2
Mean	Mn	34.0	45.3	54.7
		±1.1	±1.7	±1.3

[a] (I) Algae and fresh pond water daily;
(II) fresh pond water daily; and
(III) fresh pond water Mon., Wed., and Fri.

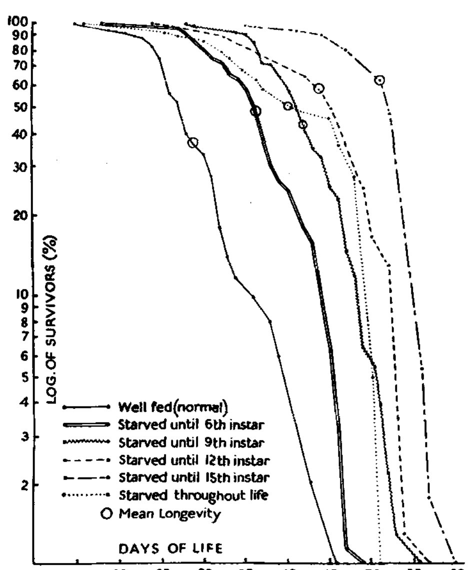


Figure 3. Effect of Restricted Food Upon the Survivorship of *Daphnia longispina.*

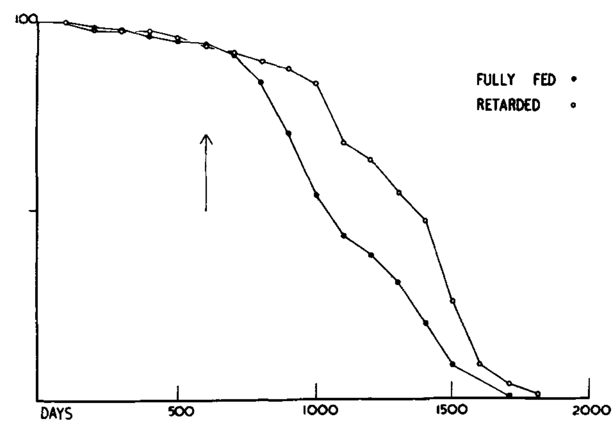


Figure 4. Survivorship Curve of Female *Lebistes reticulatus* Fed Live *Tubifex* Worms Weekly (•) or Biweekly (∘). Arrow indicates realimentation of the restricted fish. The animals were maintained at 23° C.

McCay, et al. (9,10,16), carried out a series of three studies that supported the observation that nutritional deprivation increases lifespan. Since these early studies, the increased lifespan associated with underfeeding has been reported in rats by Berg and Simms (17) (Table 2), Ross (4,18,19) (Table 3), Leveille (20) (Figure 5), and Riesen, et al. (21), and in mice by Leto, et al. (22)(Figure 6).

Table 2. The Effect of Reduced Dietary Intake on Life Span of Male Sprague-Dawley Rats

Diet [a]	N [b]	% Survivorship at 799 days	Max. Body Wt. (gm)
Ad lib	50	48	448
33% Restriction	48	87	342
46% Restriction	76	81	275

[a] Rockland "D free" pellets.

[b] Number of rate at start.

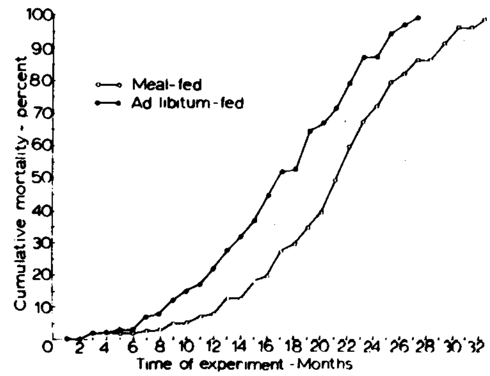


Figure 5. Cumulative Mortality for Male Sprague-Dawley Rats Offered Food for Periods of Two Hours (o——o, meal-fed) or 24 Hours (●——●, *ad libitum*-fed) Daily.

Table 3. The Effect of Dietary Intakes and Protein Levels on Life Span of Male Sprague-Dawley Rats.

	Unrestricted Dietary Intake				
Diets	Comm.	A	B	C	D
N[a]	150	25	25	25	25
Casein (%)	23	30.0	50.8	8.0	21.6
Caloric Value (Cal/gm)	3.1	4.1	4.2	4.1	4.2
Food Intake (gm/day)	25.0	17.4	18.8	15.0	19.6
Max. Body Weight (gm)	610		(not available)		
Mean Life Span (days)	730	305	595	825	600
Max. Life Span (days)	1072	347	810	1251	895

	Restricted Dietary Intake			
Diets	A	B	C	D
N[a]	150	60	150	135
Casein (%)	30.0	50.8	8.0	21.6
Caloric Value (Cal/gm)	4.1	4.2	4.1	4.2
Food Intake (gm/day)	14.3	8.5	14.3	5.3
Max. Body Weight (gm)	420	287	390	162
Mean Life Span (days	904	935	818	929

[a] Number of rate at start

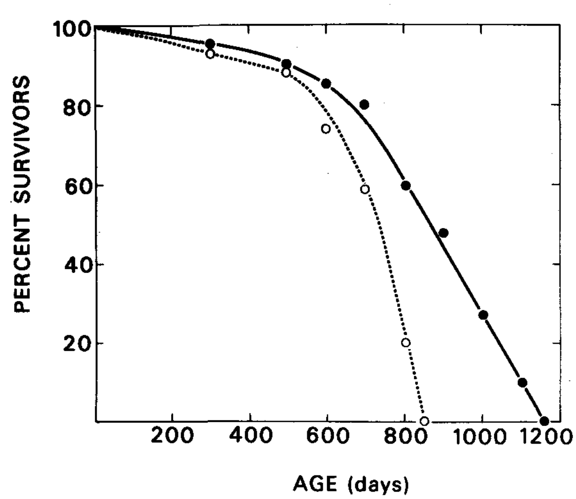


Figure 6. Survivorship Curve of Female C57BL/6J Mice Fed *ad libitum* Either a 26% Casein Diet (•) or 4% Casein Diet (o); the mean lifespans and SEMS were 685 ± 22.8 and 852 ± 27.4 days respectively.

Adult Animals. The life expectancy of adult animals can be increased by dietary manipulations as can be seen in Figure 7 (23), Table 4 (Beauchene, R. E.: Dept. of Nutrition, Univ. of Tennessee, Knoxville, Tennessee; personal communication), Table 5 (13), Table 6 (24), Table 7 (25), Table 8

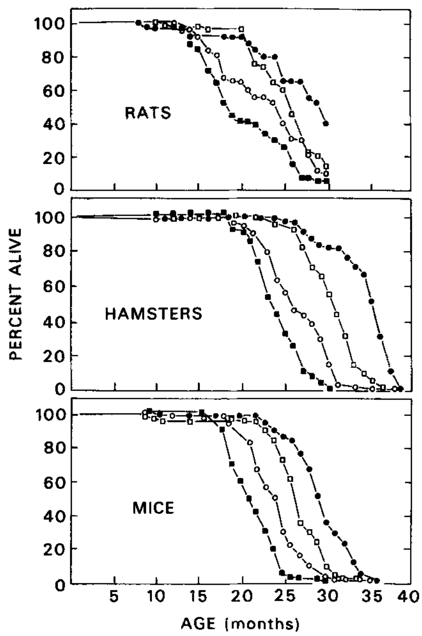


Figure 7. Effect of Various Dietary Regimens on the Survivorship of Rats, Hamsters, and Mice. Group 1 (■) was fed *ad libitum* throughout life; Group 2 (○) was fed one-half the amount of food consumed by Group 1 throughout their life; Group 3 (□) was fed *ad libitum* until one year of age and then restricted thereafter; Group 4 (•) was restricted until one year of age and then fed *ad libitum* thereafter.

Table 4. The Effect of Intermittent Feeding[a] on Life Span of Male Wistar Rats

Diet [b]	N[e]	50% survivorship (weeks)
Ad lib [c]	25	135
Ad lib-restricted[d]	30	156
Restricted-*ad lib*[d]	30	152
Restricted [c]	25	172

[a] Fed every other day.
[b] Wayne Lab Blox Diet.
[c] Throughout life span.
[d] Dietary regimen changed at one year of age.
[e] Number of rats at start.

Table 5. The Effect of Changes in Nutrition Following Cessation of Egg Production on the Life Span of Rotifer *(Philodina acuticornis)*

N [a]	Interval A-C[b]	Interval C-E[b]	Mean Life Span ± SEM (Days)
30	I [c]	I	33.4 ± 1.27
28	I	III	41.8 ± 2.62
22	III	III	53.4 ± 3.65
29	III	I	57.7 ± 1.13

[a] Number at start.
[b] A = day hatched; C = end of egg production; E = death.
[c] (I) Algae and fresh pond water daily; (III) fresh pond water Mon., Wed., and Fri., animals maintained at 25° C.

Table 6. Daily Dietary Allotments and Mortality Risk after 300 days of Age.

Diet (% casein)	Level of Allotment %	Total Food g	Casein g	Sucrose g	Corn Oil g	Total Calories Kcal	Mortality Index (x100)
Commercial [a]	100	25.0				85.51	105
	80	20.0				68.4	106
	70	17.5				59.9	*83*
	60	15.0				51.3	*79*
A	100	18.0	5.40	10.98	0.90	73.6	5550
(30.0%)	90	16.2	4.86	9.88	0.81	66.3	1180
	80	14.4	4.32	8.78	0.72	58.9	1940
	71.5	12.9	3.86	7.85	0.64	52.6	723
B	100	19.0	9.66	6.44	1.61	78.9	178
(50.9%)	78	14.8	7.53	5.02	1.25	61.4	122
	55.9	10.6	5.40	3.60	0.90	44.1	115
	40	7.6	3.86	2.58	0.64	31.6	675
C	100	15.0	1.20	12.45	0.75	61.4	2103
(8.0%)	96	14.4	1.15	11.95	0.72	58.9	2882
	86.8	13.0	1.04	10.81	0.65	53.3	3195
	79.3	11.9	.95	9.88	0.60	48.7	3195
D	100	20.0	4.32	10.81	2.70	84.8	200
(21.6%)	80.7	16.1	3.49	8.72	2.18	68.4	126
	59.6	11.9	2.58	6.44	1.61	50.6	99
	52	10.4	2.25	5.62	1.41	44.1	*68*

[a] Purina Lab Chow, 23% protein

(26), and Table 9 (3). It is obvious from these data that there are a number of inconsistencies regarding the effect of dietary restriction on the extension of life span in adult animals. Unfortunately, the limited amount of data does not allow for a critical evaluation as to the variables which may be influencing the ability of a mature organism to respond to dietary manipulation. However, the age at which dietary manipulations are initiated is important. This is clearly indicated by the data of Dunham (27) shown in Figure 8. Daphnia adequately fed up to the sixth instar, then subjected to dietary restriction, showed an increase in lifespan. However, a shortening in lifespan was observed if this dietary manipulation was imposed later in life. A shortening of lifespan due to dietary restriction imposed on 19-month-old rats has been observed by Barrows and Roeder (28). In these experiments, restricted animals were offered 50% of the diet consumed by the controls and their lifespans were 20.5 ± 0.44 and 22.3 ± 0.43 months respectively. It is also apparent from the studies of Kopec (29) and David, et al. (30) (Figure 9), that the degree of dietary restriction

Table 7. The Effect of Reduced Dietary Intake on the Mean Survival Time of Sprague-Dawley Rats

Diet [a]	Mean Survival Time (days) Males[b]	Females[b]
Ad lib	706	756
20% Restriction	856	872
40% Restriction	924	872
Ad lib for 12 weeks, 20% Restriction thereafter	801	871
Ad lib for 12 weeks, 40% Restriction thereafter	927	943
20% Restriction for 12 wks. *Ad lib* thereafter	723	788
40% Restriction for 12 wks. *Ad lib* thereafter	782	805

[a] Natural products diet: lipid, 18.5%; protein, 23%; ash, 6.2%; 4.4 kcal./gm.
[b] 50 rats at start; diets started just after weaning.

Table 8. The Effect of Various Dietary Regimens on Life Span of Female Rats

Dietary Regimen [a]	Life expectancy ± SE (days)	Significant differences
A	763 ± 94	P<0.001
B	980 ± 50	P<0.001
C	828 ± 73	
D	282 ± 40	
E	<100	

[a] A - stock diet throughout life; B - stock diet for 120 days, then 20% stock diet and 80% starch; C - 30% stock diet and 70% starch throughout life; E - protein-free diet. All diets were *ad libitum.*

Table 9. The Effect of Dietary Protein Levels on the Survival of 16-mo. Female Wistar Rats

Dietary Protein Levels (%)	N	Survival (week)
24	44	29.5 ± 2.28 [a]
12	44	37.0 ± 2.00 [b]
8	44	30.0 ± 2.30
4	44	31.6 ± 1.70

[a] Mean ± SEM
[b] p = .001

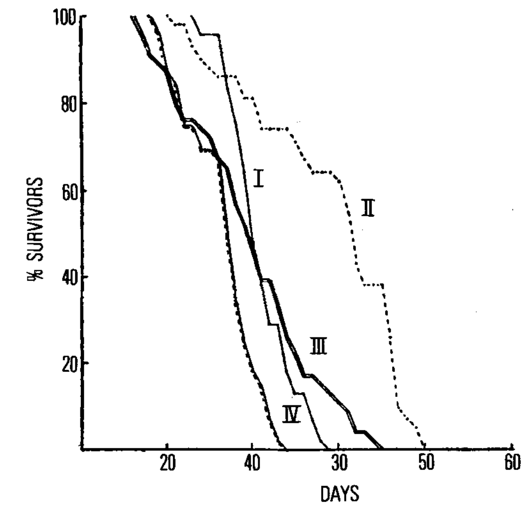


Figure 8. The Effect of Dietary Restriction on the Survivorship Curves of *D. longispina.* I - represents well-fed controls; II - semi-starved controls; III - group well-fed to sixth instar and then semi-starved; and IV - well-fed to 12th instar and then semi-starved. Semi-starvation was brought about by diluting normal medium some 30 to 40 times with pond water.

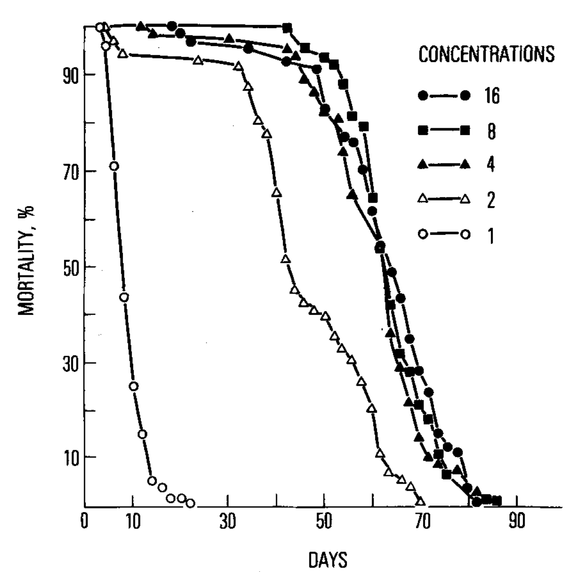


Figure 9. Survival curves of Adult Drosophila (Both Sexes). The usual axenic medium contained eight percent brewer's yeast and eight percent cornflour. This medium was diluted with an agar solution in order to produce concentrations ranging from 16 to 1 percent.

imposed on an adult organism may influence the lifespan of Drosophila.

The sex of an animal may also influence its response to dietary restriction in terms of lifespan. For example, the life shortening effect of dietary restriction in adult Drosophila (30) was more marked in males (33%) than in females (17%). In addition, the lifespan of 445-day-old female, but not male rats, was increased by decreasing the dietary protein (31). Thus, it is apparent that further studies must be carried out to define effective ways of consistently increasing the lifespan of adult organisms.

Other Variables

The preceding data suggest that life extension due to dietary restriction is observed in a variety of species and may likely represent a very basic biological process. Unfortunately, the mechanisms responsible remain unknown. However, there are a number of studies which have determined various physiological and biochemical variables, as well as disease incidence, in normal animals as well as in those whose lifespan has been increased by dietary restriction. From such data, working models may evolve which would be useful in proposing various testable hypotheses related to this phenomenon. Therefore, it would be of interest to examine these data.

Physiological Variables

Animals whose lifespan has been increased by low protein feeding (4%) (Figure 6) have a lower rectal temperature than those fed the control diet (26%) protein (22) (Figure 10). Unfortunately, little information is available on the effect of body temperature on the lifespan of homeothermic animals. Nevertheless, the lifespan of poikilothermic animals increases with decreased environmental

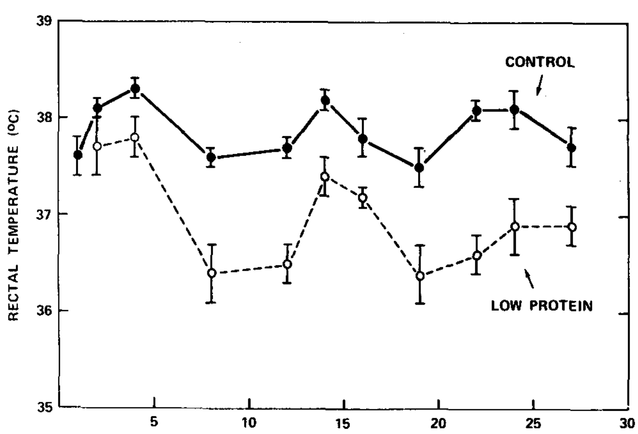


Figure 10. Effect of Low-Protein on Rectal Temperature of Female C57BL/6J Mice. Vertical bars represent SEM. The mean lifespan and SEM of the animals fed either the low-protein or control diet was 852 ± 27.4 days, and 685 ± 22.8 days, respectively.

temperature (32). It is generally assumed that this latter finding is a result of a decreased metabolic rate due to the lowering of the rates of biochemical reactions at the reduced temperature. However, the low body temperatures of these mice were associated with an increased oxygen consumption (22) (Figure 11). Furthermore, recent studies (33,34) in which poikilothermic animals have been exposed to different temperatures at various times in the life cycle, suggest a more complicated mechanism which may be independent of metabolic rate.

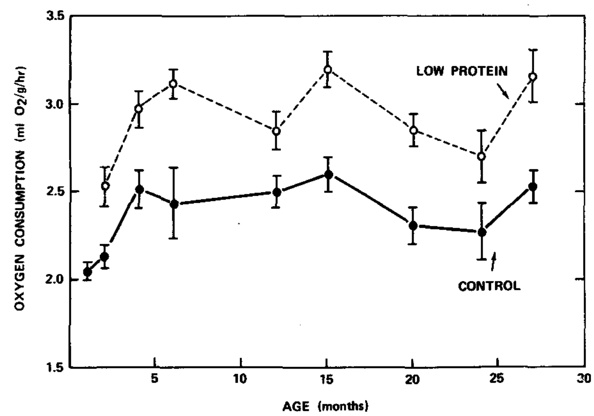


Figure 11. Effect of Low-Protein Feeding on Oxygen Consumption of C57BL/6J Female Mice. Vertical bars represent SEM. The mean lifespan and SEM of the animals fed either the low-protein or control diet was 852 ± 27.4 days and 685 ± 22.8 days, respectively.

The biological mechanism responsible for the association between the increased oxygen consumption of dietarily restricted mice and their lifespan is unknown since complete agreement on the effects of oxygen uptake on the lifespan of animals is not found in data presently available. For many years, an inverse correlation has been described among various species of mammals, i.e., the higher the oxygen uptake per unit of body weight, the shorter the lifespan (35). Indeed Kibler and Johnson (36) showed that rats exposed to cold temperatures throughout their life experienced a marked decrease in longevity and 40% increase in oxygen consumption. However, Weiss (Weiss, A. K.: Metabolism during aging in highly inbred and F_1 hybrid rats. Fed. Proc., 21:219, 1962) (37) reported that although the lifespan of the F-1 generation was longer and the BMR lower than either parental strains (AXC and Fisher), the BMR of the parents was essentially the same in spite of marked differences in longevity. Finally, Storer (38) had reported a direct relationship between oxygen consumption and lifespan among 18 strains of mice. Should the longevity of animals vary inversely with basal metabolic rate, the increased oxygen consumption due to dietary restriction would shorten lifespan, whereas should the converse relationship exist, the increased oxygen consumption of these mice would result in an increased lifespan. Therefore, these data indicating an increased oxygen consumption and reduced rectal temperature in dietarily restricted animals cannot presently contribute to our knowledge of the biological mechanism responsible for the increased lifespan.

Diseases

Although the incidence of many diseases increases with age, the relationship between disease and aging remains unknown. Data presented in Figure 12 (39) and Figure 13 (40) and in Table 10 (1), Table 11 (17) and Table 12 (41) clearly indicate that dietary restriction which increased lifespan delays the onset of a variety of diseases in mice and rats. However, the data are not consistent regarding the relationship between dietary restriction and disease incidence. Furthermore, there are not indications as to the mechanisms responsible for this delay in onset.

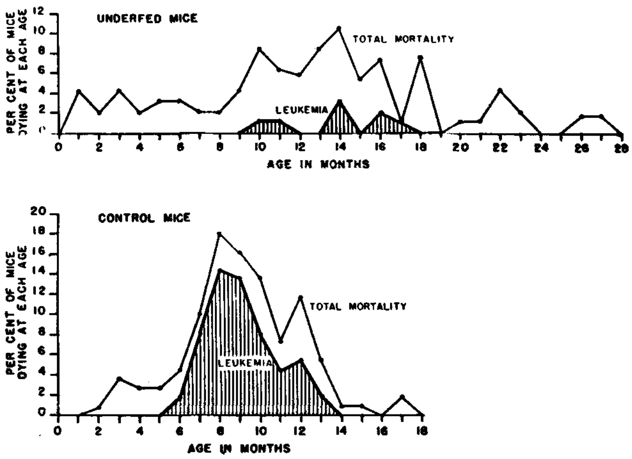


Figure 12. Effect of Underfeeding on the Mortality and Incidence of Leukemia in AK Mice. The underfed (47 males, 47 females) mice were offered 1.5 gr. of Wayne Fox Food Blox daily; controls (52 males, 59 females) were given the same diet *ad libitum*.

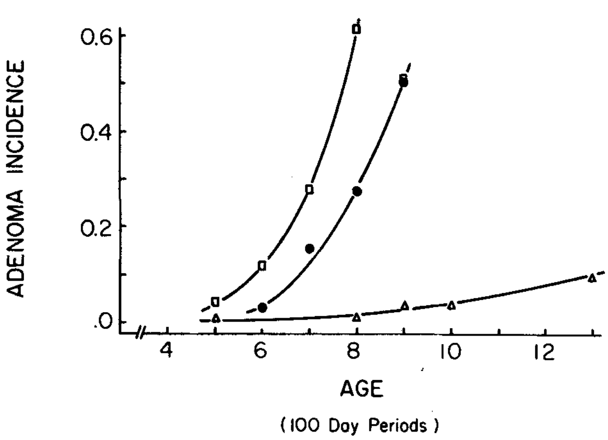


Figure 13. Influence of Dietary Regimen on the Incidence of Adenomas in Male, COBS (Charles River) Rats. (□) Rats fed *ad libitum* throughout postweaning life; (Δ) rats fed a restricted amount of diet throughout postweaning life; (•) rats fed a restricted amount of diet 21-70 days of age, and then fed *ad libitum*. Composition of diet: casein, 22.0%; sucrose, 58.5%; Mazola oil, 13.5%; salt mixture (USP XII), 6.0%; vitamins, and trace elements.

Biochemical Variables

It has been proposed (3) that reduced protein

Table 10. The Effect of Dietary Restriction on the Incidence of Spontaneous Mammary Carcinoma and the Survival of C_3H Mice.

	Restricted	Unrestricted
N [a]	44	51
Caloric Intake/day [b]	8.4	11.5
Protein Intake/day [b]	0.64	0.65
Max. body weight, grains	15.5	32.0
% Survival at 16 months	57.0	29.0
Cumulative tumors[c] (%) at 16 months	0	63.0

[a] Number of mice at start.
[b] After 100 days of age.
[c] Spontaneous mammary carcinoma.

Table 11. The Effect of Dietary Restriction on the Incidence of Three Major Diseases in Male Sprague-Dawley Rats.

		% Incidence [b]		
	N[a]	Glomerular-nephritis	Periarteritis	Myocardial degeneration
Unrestricted	24	100	63	96
33% Restricted	42	36	17	28
46% Restricted	38	13	3	24

[a] Number of rats at start.
[b] At 800 days of age.

Table 12. Progressive Glomerulonephrosis Index of Male Sprague-Dawley Rats Fed Semisynthetic Diets

	Number of Cases		Disease
Dietary Groups[a]	Expected	Observed	Index[b]
A	186.4	46	24.7
B	88.2	1	1.1
C	152.5	16	10.5
D	10.5	4	1.9

[a] The intakes of animals fed diet A, B, C, or D were restricted (See Table 3).
[b] Computed from rats dying from natural death only. Disease index expressed as percentage (computed as number of actual against expected cases). Expected cases equals disease rate at each age period of "control" population times exposure of experimental population. A value of the index of less than 100 indicates a beneficial effect of the experimental diet.

synthesis may increase lifespan by retarding genetic informational transfer during early life and reducing the use of the genetic code and thereby minimizing genetic imperfections as they may occur in late life. The following studies support the concept that reduced protein synthesis retards genetic informational transfer during early life. In the first study, Table 13 (3), development and growth were reduced by the administration of cycloheximide, an inhibitor of protein synthesis, into one-day-old chick embryos. In the second study, enzymatic activities were determined throughout the lifespan of normal rotifers as well as those whose lifespan was increased by dietary restriction; Figure 14-16 (13). Enzymatic activities were considered adequate expressions of genetic program on the basis that under all conditions, the following always occurred; 1) the patterns of age change in the enzymatic activities were similar; 2) the maximal levels of activity were similar; and 3) age-dependent decreases in the ratio of malate dehydrogenase (MDH) to lactate dehydrogenase (LDH), always occurred. Similar data have been reported by Ross

Table 13. The Effect of Cycloheximide on the Development of Chick Embryos

Days		Length (mm)	Number of Somites	Stage of Development[a]	Heart Rate Beats/ min.
0.8 Micrograms Cycloheximide[b]					
2	Con	4.6 ± 0.15[c]	17.0 ± 0.6	13.4 ± 0.3	15.0 ± 3.5
	Exp.	4.1 ± 0.15	15.2 ± 0.6	11.8 ± 0.3	9.8 ± 2.8
	P	.01	.05	.01	NS
3	Con	6.2 ± 0.15		17.1 ± 0.1	38.2 ± 5.0
	Exp.	5.7 ± 0.20		10.8 ± 0.1	26.8 ± 5.5
	P	.05		.05	NS
1.0 Micrograms Cycloheximide[b]					
2	Con	4.3 ± 0.15	16.5 ± 0.7	12.6 ± 0.2	15.0 ± 2.0
	Exp.	3.5 ± 0.23	12.7 ± 1.3	10.8 ± 0.5	7.0 ± 1.0
	P	.001	.01	.001	.001
3	Con	5.8 ± 0.15		16.2 ± 0.2	40.0 ± 8.0
	Exp.	5.3 ± 0.10		15.2 ± 0.2	29.0 ± 4.0
	P	.01		.001	NS

[a] Hamburger, V., and Hamilton, H. L.: A series of normal stages in the development of the embryo. J. Morph., 88:49-92, 1951.
[b] Injected into one-day-old embryos.
[c] Mean ± SEM.

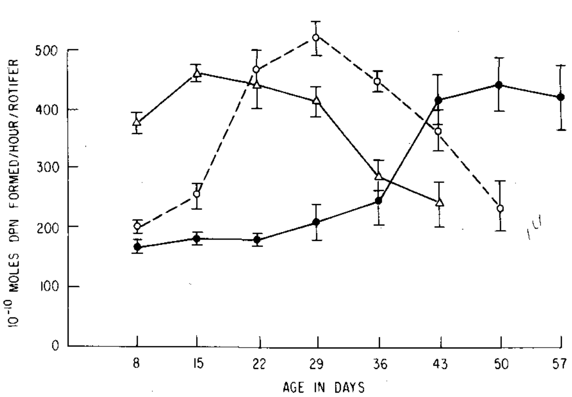


Figure 14. Effect of Nutrition on Malic Dehydrogenase Activity in Rotifers (*Philodina acuticornis*). (Δ) Diet I (algae and fresh pond water daily; mean lifespan = 34.0 ± 1.1 days); (○) Diet II (fresh pond water daily; mean lifespan = 45.3 ± 1.7 days); and (•) Diet III (fresh pond water Mon., Wed., and Fri.; mean lifespan = 54.7 ± 1.3 days). Vertical bars represent SEM.

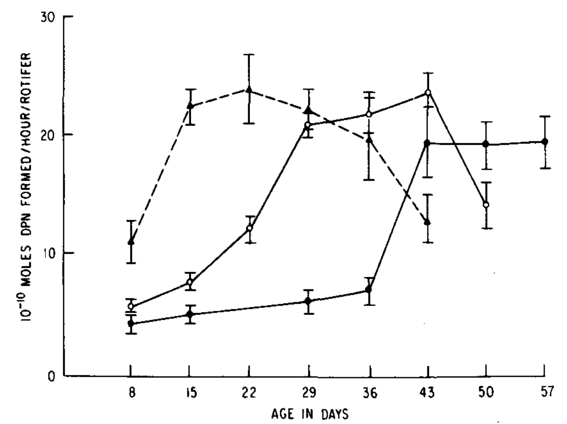


Figure 15. Effect of Nutrition of Lactic Dehydrogenase Activity in Rotifers (*Philodina acuticornis*). (▲) Diet I (algae and fresh pond water daily; mean lifespan = 34.0 ± 1.1 days); (○) Diet II (fresh pond water daily; mean lifespan = 45.3 ± 1.7 days); and (•) Diet III (fresh pond water Mon., Wed., and Fri.; mean lifespan = 54.7 ± 1.3 days). Vertical bars represent SEM.

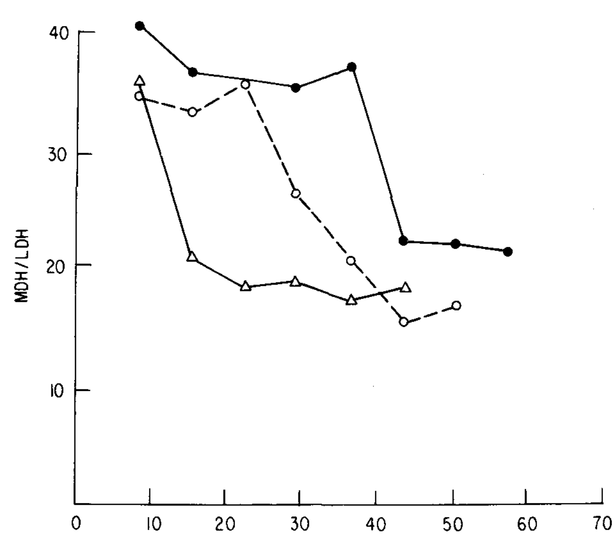


Figure 16. Effect of Nutrition on MDH/LDH. (Δ) Diet I (algae and fresh pond water daily; mean lifespan = 34.0 ± 1.1 days); (○) Diet II (fresh pond water daily; mean lifespan = 45.3 ± 1.7 days); and (•) Diet III (fresh pond water Mon., Wed., and Fri; mean lifespan = 54.7 ± 1.3 days).

(18) for the enzymes adenosine triphosphatase and alkaline phosphatase in the livers of rats (Figure 17).

Studies in which enzymatic activities of the tissues of normal and dietarily restricted mice and rats based on DNA (Figures 18-20) (42) or numbers of hepatocytes (Figure 21) (19, Table 10) suggest a reduced use of the genetic code throughout lifespan. This suggestion is based on the premise that reduced enzymatic activity per DNA or per cell represents a reduced enzyme synthesis. This premise is supported

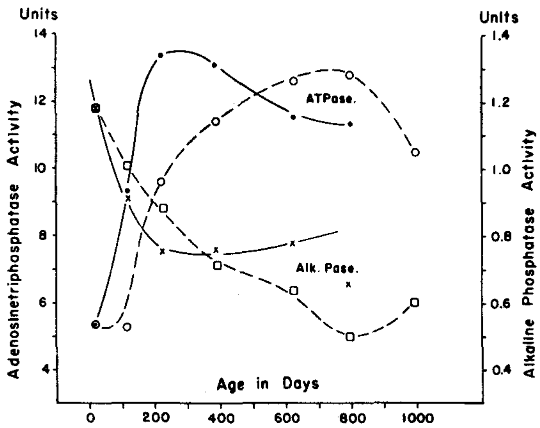


Figure 17. Effect of Diet and Age on the Activity of Hepatic Adenosinetriphosphatase and Alkaline Phosphatase in Male Sprague-Dawley Rats. Enzymatic activities are expressed as activity per milligram wet weight of tissue. Rats maintained on commercial diet *ad libitum:* (x) Alakaline phosphatase activity; (•) Adenosinetriphosphatase activity. Rats whose daily food allotment of Diet C was restricted (see Table III): (□) Alkaline phosphatase activity; (○) Adenosinetriphosphatase activity.

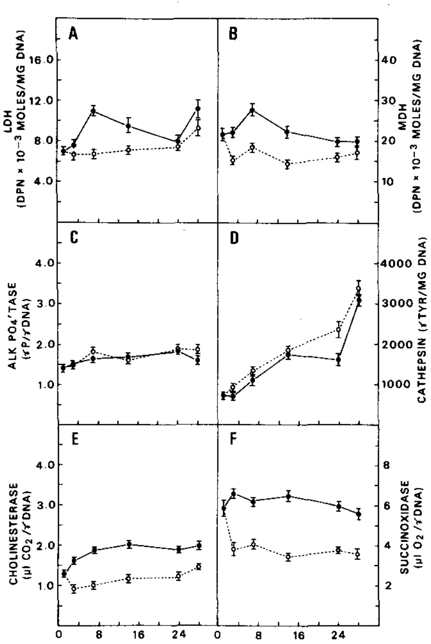


Figure 18. Effect of Age and Diet on the Enzymatic Activities of Liver of Female C57BL/6J Mice Fed (•) 26% Casein Diet or (○) 4% Casein Diet. Vertic bars represent SEM. The mean lifespan and SEM of the animals fed either the low-protein or control diet was 852 ± 27.4 days and 685 ± 22.8 days respectively.

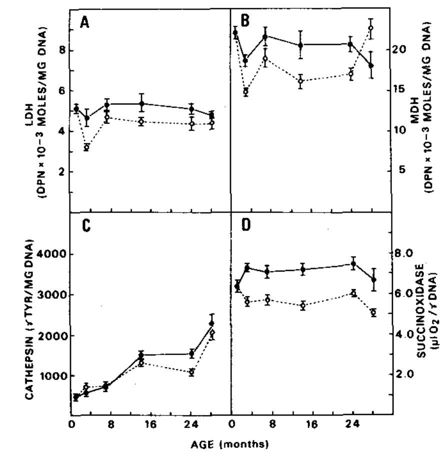


Figure 19. Effect of Age and Diet on the Enzymatic Activities of Kidneys of Female C57BL/6J Mice Fed (•) 26% Casein Diet or (○) 4% Casein Diet. Vertical bars represent SEM. The mean lifespan and SEM of the animals fed either the low-protein or control-diet was 852 ± 27.4 days and 685 ± 22.8 days respectively.

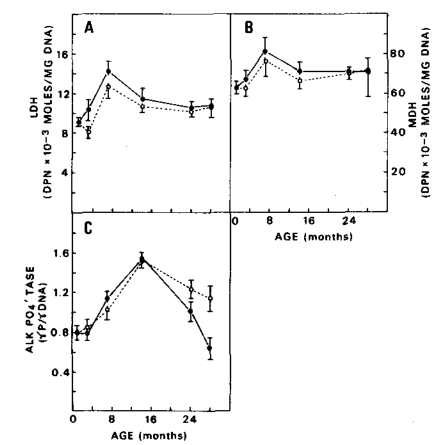


Figure 20. Effect of Age and Diet on the Enzymatic Activities of Hearts of Female C57BL/6J Mice Fed (•) 26% Casein Diet or (○) 4% Casein Diet. Vertical bars represent SEM. The mean lifespan and SEM of the animals fed either the low-protein or control diet was 852 ± 27.4 days and 685 ± 22.8 days respectively.

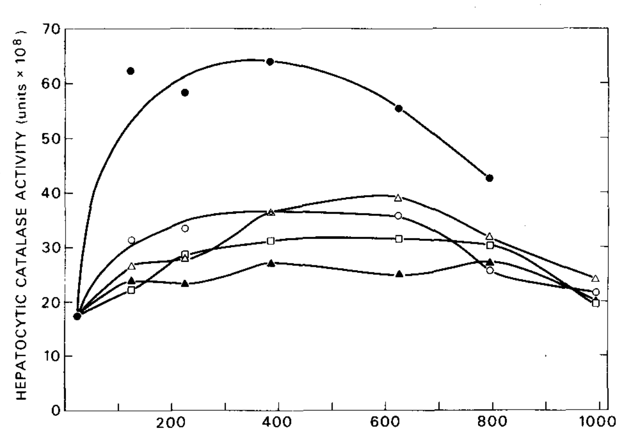


Figure 21. Effect of Diet and Age on the Activitiy of Hepatic Catalase in Male Sprague-Dawley Rats. Rats maintained on commercial diet *ad libitum* (•); rats whose daily food allotment was restricted (see Table III): (○) Diet A; (Δ) Diet B; (□) Diet C; (▲) Diet D.

by the work of Schimke (43) in which rats were fed different dietary protein levels and the total amount of liver arginase and its rates of synthesis and degradation were measured. These data are shown in Figure 22. It is apparent that animals in a steady state, being fed a diet containing 70% casein, had a total liver arginase of 9 mg. and the protein synthesis

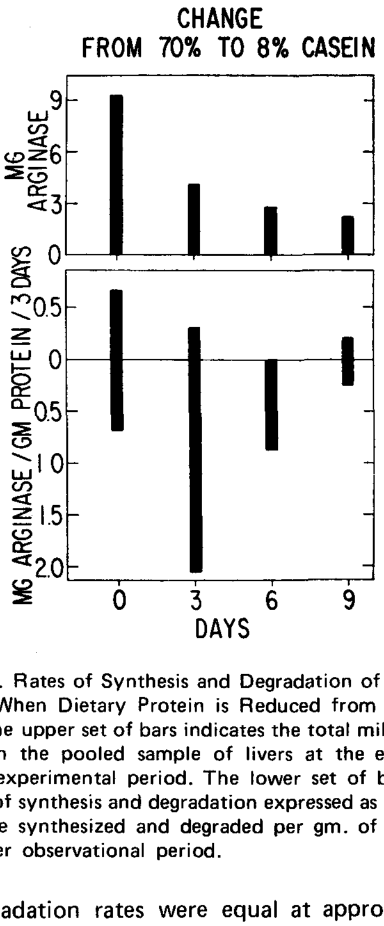


Figure 22. Rates of Synthesis and Degradation of Rat Liver Arginase When Dietary Protein is Reduced from 70 to 8% Casein. The upper set of bars indicates the total milligrams of arginase in the pooled sample of livers at the end of the specified experimental period. The lower set of bars shows the rates of synthesis and degradation expressed as milligrams of arginase synthesized and degraded per gm. of total liver protein per observational period.

and degradation rates were equal at approximately 0.7 mg. arginase/gr. protein/three days. Following the ingestion of a diet containing 8% casein, the rate of degradation was increased while the rate of synthesis and the total liver arginase decreased during the first six days of feeding. By the ninth day, the animals had reduced their total arginase to approximately 2 mg. and the rates of both synthesis and degradation had decreased to approximately 0.2 mg. arginase/gr. protein/three days. Therefore, it is apparent that under steady state conditions, i.e., when the rates of synthesis and degradation are equal and constant, a reduction in cellular enzymatic activity associated with reduced dietary protein is likewise associated with a reduction in the rate of protein synthesis. It seems reasonable therefore to assume that this reduced rate of protein synthesis is associated with a reduced use of the genetic code.

In a more recent series of studies (44), this hypothesis was further investigated by comparing enzymatic activities of mice fed two dietary regimes reported to increase lifespan; namely low protein (4,42) and intermittent feeding (45,46) (Beauchene, R. E.: Dept. of Nutrition, Univ. of Tennessee, Knoxville, Tennessee; personal communication). Twenty-one-day-old and 17-month-old female mice were fed the following three dietary regimes for one to six months: 1) 24% protein *ad libitum*; 2) 4% protein *ad libitum*; or 3) 24% protein intermittently fed (diet offered for 24 hours on Monday and Wednesday, and for eight hours on Friday). Those animals referred to as intermittent-fed were sacrificed either on Tuesdays or Thursdays, i.e., following a 24-hour feeding period. Those referred to as intermittent-fasted were sacrificed either on Wednesdays or Fridays, i.e., following a 24-hour fasting period. On the assumption that an increase in the DNA per unit wet weight represents a decrease in the size of cells, then differences in the concentration of DNA in the livers and kidneys (Table 14) indicated that fasting or feeding a 4% diet *ad libitum* resulted in small cells and that cells increased in size during a period of refeeding. In addition, the data indicated that a reduction in cell size was accompanied by reduced cellular protein. These changes in cellular protein were likewise approximated by changes in cellular enzymatic activities of succinoxidase, cholinesterase, and malic dehydrogenase calculated

Table 14. Concentration of Protein and DNA in the Livers and Kidneys of Female Mice Fed Different Dietary Regimes

	24% Protein	4% Protein	Intermittent[a] Fed	Intermittent[a] Fasted
		Liver		
DNA				
γ/mg. tissue				
2 mo. old[d]	3.52±0.08[b]	4.04±0.07[c]	3.19±0.09[c]	4.16±0.19[c]
7 mo. old[d]	4.13±0.19	4.55±0.17	3.49±0.11[c]	5.24±0.18[c]
22 mo. old[e]	3.64±0.16	4.43±0.16	3.35±0.13	-----
Protein				
mg./gr. tissue				
2 mo. old	191.3±3.3	166.1±4.4[c]	203.5±6.2	212.1±5.0
7 mo. old	191.7±6.1	157.2±3.5[c]	190.0±4.2	206.3±8.0
22 mo. old	182.1±4.3	158.2±5.3[c]	191.0±9.3	-----
mg./mg. DNA				
2 mo. old	54.7±1.7	41.1±0.8[c]	63.8±0.6[c]	51.6±1.6
7 mo. old	46.7±0.9	34.8±1.1[c]	54.8±1.8[c]	39.6±1.9[c]
22 mo. old	51.0±2.9	36.2±1.8[c]	57.8±4.6	-----
		Kidney		
DNA				
γ/mg. tissue				
2 mo. old	6.46±0.33	8.08±0.13[c]	5.89±0.11	6.45±0.15
7 mo. old	6.63±0.18	8.82±0.18[c]	6.00±0.11[c]	7.36±0.18[c]
22 mo. old	6.34±0.18	6.97±0.25[c]	5.41±0.07[c]	-----
Protein				
mg./gr. tissue				
2 mo. old	173.0±6.3	178.8±5.6	193.4±3.3[c]	173.1±3.7
7 mo. old	172.6±3.8	179.7±3.7	163.5±2.9	171.4±5.0
22 mo. old	163.4±2.9	156.4±3.8	159.6±3.0	-----
mg./mg. DNA				
2 mo. old	27.4±1.7	22.1±0.5[c]	33.0±1.0[c]	26.8±0.6
7 mo. old	26.2±0.8	20.4±0.6[c]	27.3±0.5	23.4±0.9[c]
22 mo. old	25.9±0.8	22.5±0.5[c]	29.5±0.4[c]	-----

[a] Fed *ad libitum* a 24% protein diet on Mon., Wed., and Fri.

[b] Mean ± SEM.

[c] $p < .05$ when compared to values obtained for animals fed the 24% protein diet.

[d] $C_{57}BL/6J$ mice fed dietary regimes from weaning.

[e] CBA mice fed dietary regimes from 17 months of age.

on the basis of DNA in liver (Table 15) and kidney (Table 16). The activities of these three enzymes were decreased in the 4% and intermittent-fasted animals and increased in the intermittent-fed animals. Furthermore, the mean values of the enzymatic activities per mg. of DNA of the intermittent-fed and intermittent-fasted animals were essentially the same

Table 15. The Effect of Various Dietary Regimes on Enzymatic Activities in Livers of Female Mice.

	Protein	Protein	Intermittent[a] Fed	Intermittent[a] Fasted
Malic dehydrogenase (millimoles DPNH/hr./mg. DNA)				
2 mo. old[d]	10.211±0.293[b]	7.344±0.148[c]	11.578±0.178[c]	10.371±0.506
7 mo. old[d]	7.998±0.343	5.543±0.383[c]	9.714±0.409[c]	7.009±0.218[c].
22 mo. old[e]	8.377±0.601	6.427±0.404[c]	9.916±0.778	----
Succinoxidase ($u1O_2$/hr./γDNA				
2 mo. old	6.71 ± 0.37	4.00 ± 0.12[c]	7.53 ± 0.20	6.73 ± 0.21
7 mo. old	5.96 ± 0.26	3.21 ± 0.16[c]	6.99 ± 0.22[c]	5.87 ± 0.20
22 mo. old	7.29 ± 0.32	3.39 ± 0.22[c]	7.41 ± 0.47	----
Cholinesterase ($u1CO_2$/hr./γDNA)				
2 mo. old	7.34 ± 0.31	5.02 ± 0.30[c]	9.79 ± 0.49[c]	6.36 ± 0.32[c]
7 mo. old	6.82 ± 0.44	3.95 ± 0.32[c]	8.47 ± 0.27[c]	6.06 ± 0.31
22 mo. old	6.29 ± 0.46	4.69 ± 0.31[c]	8.35 ± 0.83[c]	----

[a] Fed *ad libitum* a 24% protein diet on Mon., Wed., and Fri.
[b] Mean ± SEM.
[c] p<05 when compared to values obtained for animals fed the 24% protein diet.
[d] C_{57}BL/6J mice fed dietary regimes from weaning.
[e] CBA mice fed dietary regimes from 17 months of age.

Table 16. The Effect of Various Dietary Regimes on Enzymatic Activities in the Kidneys of Female Mice.

	Protein	Protein	Intermittent[a] Fed	Intermittent[a] Fasted
Malic dehydrogenase (millimoles DPNH/hr./mg. DNA)				
2 mo. old[d]	9.156 ± 0.632	6.956 ± 0.271[c]	9.353 ± 0.389	9.455 ± 0.284
7 mo. old[d]	8.055 ± 0.322	5.917 ± 0.147[c]	8.968 ± 0.342	7.282 ± 0.261
22 mo. old[e]	8.006 ± 0.431	6.562 ± 0.406[c]	8.961 ± 0.355	----
Succinoxidase ($u1O_2$/hr./γDNA				
2 mo. old	7.55 ± 0.38	5.34 ± 0.20[c]	7.26 ± 0.41	6.60 ± 0.19[c]
7 mo. old	6.78 ± 0.25	5.24 ± 0.15[c]	7.90 ± 0.20[c]	6.72 ± 0.12
22 mo. old	5.89 ± 0.28	4.83 ± 0.20[c]	6.45 ± 0.29	----
Cholinesterase ($u1CO_2$/hr./γDNA)				
2 mo. old	7.34 ± 0.31	5.02 ± 0.30[c]	9.79 ± 0.49[c]	6.36 ± 0.32[c]
7 mo. old	6.82 ± 0.44	3.95 ± 0.32[c]	8.47 ± 0.27[c]	6.06 ± 0.31
22 mo. old				

[a] Fed *ad libitum* a 24% protein diet on Mon., Wed., and Fri.
[b] Mean ± SEM.
[c] p<05 when compared to values obtained for animals fed the 24% protein diet.
[d] C_{57}BL/6J mice fed dietary regimes from weaning.
[e] CBA mice fed dietary regimes from 17 months of age.

as that of the 24% *ad libitum* controls. Therefore, these data did not indicate the existence of a common biochemical alteration to explain the phenomenon of increased lifespan due to dietary restriction nor did the data obtained on intermittent feeding support the hypothesis that dietary restriction increases lifespan by reducing protein synthesis and consequently reducing use of the genetic code. However, it is obvious that marked variations in the cellular enzymatic activities of the animals subjected to intermittent feeding occur during the 48-hour interval of fasting and refeeding. These data were obtained at only two points during this time interval, and therefore do not necessarily represent the integrated cellular enzymatic activity. As final proof of this hypothesis, studies such as these must include measurements of rates of protein synthesis.

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