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Influence of Diet on Fatal Neoplastic Disease in Male Fischer 344 Rats

Isao Shimokawa,[1] Byung Pal Yu,[2] and Edward J. Masoro[2]

[1]First Department of Pathology, Nagasaki University Medical School, Japan.
[2]Department of Physiology, The University of Texas Health Science Center at San Antonio.

The influence of dietary manipulations on fatal neoplasms in male Fischer 344 rats was assessed. Particular attention was paid to leukemia and pituitary adenoma because they are the most common potentially fatal neoplasms that occur in this rat strain. The only dietary manipulation which depressed the mortality rates due to neoplastic disease was that involving a reduction in energy intake. Reduction of mineral or protein or fat intake without a reduction of energy had, at most, marginal effects on fatal neoplastic disease.

RESTRICTION of food intake of rodents below that of ad libitum fed animals (called "food restriction" or "dietary restriction") reduces the prevalence of neoplastic diseases and/or delays their appearance. The extensive literature on this subject has been comprehensively reviewed by Weindruch and Walford (1987). However, these reports have not directly addressed the influence of dietary restriction on the onset or the progression of spontaneous neoplasms or the relation of neoplastic disease to the life span of rodents.

Data from sacrificed rodents can provide useful information on age and the incidence of neoplasms, but not on the relationship of disease to life span. Moreover, the number of rats sacrificed for this purpose in most studies has been too small for adequate statistical analysis.

Data obtained from rodents which have died spontaneously may contain appreciable biases. For example, rodents die of various causes, neoplastic and non-neoplastic. If a dietary regimen modulates only the onset and the progression of non-neoplastic diseases, the difference in prevalence at death of a neoplastic disease may not relate to an influence of the diets on neoplastic disease but rather to the difference in length of life due to an action on non-neoplastic disease. Peto et al. (1980) and Gart et al. (1986) have discussed in detail the biases due to intercurrent mortality, i.e., deaths due to causes other than the particular neoplasm type being analyzed, in long-term animal study.

Pathological data of male Fischer 344 rats under various dietary regimens have been published from our laboratory (Maeda et al., 1985; Iwasaki et al., 1988a, 1988b; Masoro et al., 1989). However, the data on neoplastic diseases were not analyzed in regard to onset or progression of the disease or the effect on mortality. The purpose of this study is to evaluate the effects of dietary modulations on the neoplastic disease processes in these regards by reanalyzing these published data, applying the statistical method of Peto et al. (1980). This method circumvents the bias due to the intercurrent mortality classifying neoplastic lesions in terms of contribution to death.

With this method, the role of specific dietary components (fat, protein, mineral, and energy) in the action of dietary restriction on the development of spontaneous neoplasms is addressed. There have been a number of reports which indicate effects of specific dietary components on experimental carcinogenesis (Newberne et al., 1989). However, to our knowledge, only Ross and Bras (1965) and Carroll and Khor (1975) have published data on the influence of dietary protein and fat on spontaneously occurring neoplasm in rodents. The studies on the dietary regimen conducted in our laboratory are suitable for this analysis, because a semisynthetic base diet was used and specific components were individually restricted without energy restriction.

Materials and Methods

Source of data. — The data used in this analysis were derived from the studies reported by Yu et al. (1985), Maeda et al. (1985), Iwasaki et al. (1988a, 1988b), and Masoro et al. (1989). In these reports longevity characteristics and pathological findings were presented. Autopsy records and histopathological specimens of these rats were reviewed again for this study. The number of rats analyzed in each dietary group are summarized in Table 1.

Rat maintenance and dietary manipulations. — Rat maintenance and dietary manipulations have been fully described in previous papers (Yu et al., 1985; Iwasaki et al., 1988b). Briefly, specific pathogen-free male Fischer 344 rats were purchased as weanlings (aged 26–30 days) from the Charles River Laboratories at Kingston, NY. The care and use of the rats were in accord with the guidelines of the University of Texas Health Science Center. Until 6 weeks of age all rats were fed a standard semisynthetic diet ad libitum. The control rats continued to receive that diet ad libitum throughout life; the control rats studied between 1979 and 1983 are designated Group AL I, and those studied between 1983 and 1987 designated Group AL II. The other rat groups were switched to their respective diets at 6 weeks of age. Group ProR was fed ad libitum a low protein diet between 1979 and 1983 which reduced protein intake to about 60% of the AL groups. Groups FatR and MinR were fed ad libitum diets low in fat and minerals respectively between 1983 and 1987

Table 1. Number of Rats Examined in This Study

Dietary Group	Spontaneous Deaths[a]	Deaths Due to Neoplasms
FatR	71	30
ProR	69	24
MinR	69	32
AL I	71	16
AL II	111	32
DR II	89	46

[a]Includes a few moribund rats.

Table 2. Composition of the Diets

Component (Grams/100g of diet)	Dietary Group AL I AL II	DR	FatR	ProR	MinR
Casein (Vitamin free)	21	21	21	12.6	21
D,L Methionine	0.15	0.15	0.15	0.09	0.15
Dextrin	43.65	42.19	47.65	52.11	45.65
Sucrose	15	15	15	15	15
Corn oil	10	10	6	10	10
Ralston-Purina vitamin mix[a]	2	3.33	2	2	2
Choline Chloride	0.2	0.33	0.2	0.2	0.2
Ralston-Purina mineral mix[a]	5	5	5	5	3
Fiber[b]		3	3	3	3
Physiologic caloric value (Kcal/g)	4.10	4.04	3.97	4.10	4.18

[a]The details of the composition of the mineral mix and vitamin mix were reported previously (Bertrand et al., 1980).

[b]Solka Floc, James River Corp., Berlin, NH.

which reduced the intake of fat or mineral to about 60% of the AL group. Group DR was restricted to about 60% of the ad libitum energy intake between 1983 and 1987. The composition of the diets is reported in Table 2.

The mean food intake per rat (Kcal per day), the body weight, and the growth pattern for each of the dietary groups were fully described in previous articles (Yu et al., 1985; Iwasaki et al., 1988b; Masoro et al., 1989). The body weight showed gradual increase until 18 months of age; the weight then decreased slightly in AL I, while it leveled off until 24 months and then decreased in AL II. The food intake, the body weight, and the growth pattern of ProR corresponded to those of AL I until 18 months of age; then differed slightly from those of AL I. FatR and MinR did not differ significantly in food intake, body weight, and growth pattern from AL II through 24 months of age.

Procedures for pathological examination. — Procedures for pathological examination of spontaneously dying rats have been described in previous papers (Maeda et al., 1985; Iwasaki et al., 1988a). Briefly, the brain, heart, lungs, trachea, aorta, esophagus, stomach, small intestine, colon, liver, pancreas, spleen, kidneys, urinary bladder, prostate, testes, thyroid gland, adrenal glands, parathyroid glands, psoas and thigh muscle, sternum, femur, and vertebrae were examined histologically. Any other organs or tissues with gross lesions were also examined. It should be emphasized that almost all pituitary glands were examined histologically in AL II, DR, FatR, and MinF, while only pituitary glands with gross lesions were examined histologically in AL I and ProR.

In this study, the diagnosis of leukemia was reexamined in all the groups, because there was noticeable discrepancy in the prevalence of leukemia between our published data and the studies discussed by Stromberg and Vogtsberger (1983). They reported a prevalence of leukemia in Fischer 344 rats ranging from 10 to 35% compared to the 2.8% reported by Maeda et al. (1985) and 6.3% reported by Iwasaki et al. (1988a, 1988b) in the ad libitum fed male Fischer 344 rats maintained in our laboratory.

For this reexamination, microscopic slides were reviewed again by a pathologist (I.S.) without referring to dietary groups, age, or previous diagnoses. The early stage of leukemia was based on the criteria described by Losco and Ward (1984): depletion of small lymphocytes from the splenic white pulp, severe congestion of the red pulp, and atypical lymphocytes in the liver sinusoids or lung capillaries. In this assessment, the infiltration of atypical lymphocytes into liver sinusoids or the lung capillaries or those of other organs was considered to be pathognomonic for leukemia. Cases in which the only occurrence was depletion of small lymphocytes from the splenic white pulp, or severe congestion of the red pulp, were not diagnosed as leukemia.

Reclassification of neoplastic lesions. — All types of neoplasms observed in rats at autopsy were classified into four categories by the criteria of Peto et al. (1980): (1) incidental; (2) probably incidental; (3) probably fatal; (4) fatal. A neoplastic lesion that either directly or indirectly killed a rat was classified into Category 3 or 4, and considered as a fatal neoplasm. Neoplastic lesions which were considered not to be involved in death were classified in Category 1 or 2 and called incidental neoplasms. The classification was performed by a pathologist (I.S.) not only without reference to the dietary groups and age of rats, but also independently of the previous study teams. In addition, the classification was done without knowing the severity of non-neoplastic diseases.

Statistical analyses. — Conditional survival curves if non-neoplastic fatal lesions were eliminated were generated by Kaplan-Meier method, using the data on the neoplasms classified as fatal (Gross and Clark, 1975). In this analysis, it is assumed that rats die from either a neoplastic or a non-neoplastic cause and that each cause occurs in rats independently. It is also assumed that the cause of death in each rat can be definitively determined. Thus, spontaneous death from non-neoplastic lesion is considered to be the same censorship as random sacrifice of rats. The survival distributions were compared by Gehan-Wilcoxon test (Gross and Clark, 1975). In addition, age-specific mortality rates for neoplastic diseases in each group were also calculated from data on rats bearing fatal neoplasms.

Dietary influence on the incidence of leukemia and pituitary adenoma was tested by the method of Peto et al. (1980). This method analyzes the death rate and prevalence of a neoplasm separately to correct the intercurrent mortality,

using rats bearing the neoplasm in the fatal category and rats bearing the neoplasm in the incidental category. Then, the method finally combines these two analyses to test for a difference in incidence of the neoplasm between two groups by the chi-square statistics.

In the death rate analysis, a longevity-corrected expected number of rats bearing leukemia or pituitary adenoma in the fatal category was calculated in each dietary group relative to the control group AL I or AL II, using rats that died spontaneously. Rats in which leukemia or pituitary adenoma was found in the incidental category were treated exactly as all other rats that did not die of leukemia or pituitary adenoma. In the prevalence analysis, a longevity-corrected expected number of rats bearing leukemia or pituitary adenoma in the incidental category was also calculated, using only rats that died spontaneously. Rats in which leukemia or pituitary adenoma was found in the fatal category were excluded from the analysis. Then, these observed and expected numbers derived from the death rate and the prevalence analyses were summed to obtain "O/E ratio" which Peto et al. referred to as "relative cancer onset rate"; and also, the chi-square statistics for a test for a difference in incidence of the neoplasm between two groups were calculated. The O/E ratio is not a real onset rate; rather, this is a descriptive index to know whether or not a dietary modulation has any influence on the occurrence of a neoplasm. In this study, the O/E ratio was termed the relative onset rate for convenience, following the practice of Peto et al. (1980).

RESULTS

Conditional survival curves and mortality rates of all fatal neoplasms. — Conditional survival curves based on eliminating non-neoplastic causes of death are presented in Figure 1. Groups ProR, FatR, and MinR had survival curves which were not statistically different from those of the control groups AL I and AL II. However, the survival curve of Group DR was significantly different from that of Group AL II ($p < .0001$); i.e., the age of death due to fatal neoplasms was delayed by food restriction which included energy restriction. Age-specific mortality rates due to neoplasms are presented in Figure 2. Only Group DR differs in the mortality rates from the control group. Groups ProR, FatR, and MinR show very similar mortality rates to the control group.

The relative onset rates of leukemia and pituitary adenoma. — Tables 3 and 4 contain data on the observed (O) number and the expected (E) number of rats bearing leukemia or pituitary adenoma respectively and the O/E ratio

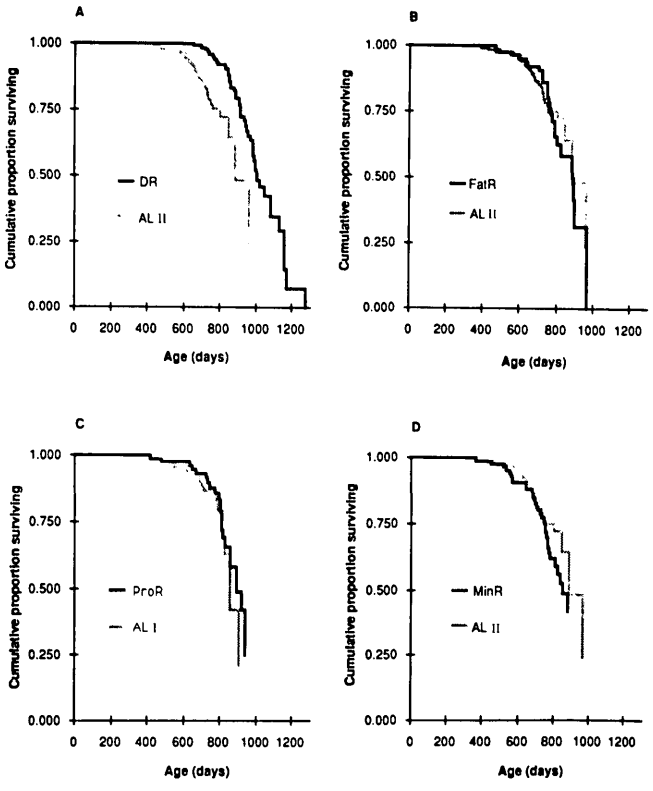

Figure 1. Conditional survival curves based on eliminating non-neoplastic cause of death in rats. A. Comparison of rats fed standard semisynthetic diet (Group AL II) and rats (Group DR) fed 60% of the mean dietary and energy intake of the ad libitum fed rats; B. Comparison of rats fed standard semisynthetic diet (Group AL II) and rats (Group FatR) fed a diet resulting in a reduced fat intake but no reduction in energy intake; C. Comparison of rats fed standard semisynthetic diet (Group AL I) and rats (Group ProR) fed a diet resulting in a reduced protein intake but no reduction in energy intake; D. Comparison of rats fed standard semisynthetic diet (Group AL II) and rats (Group MinR) fed a diet resulting in a reduced mineral intake but no reduction in energy intake.

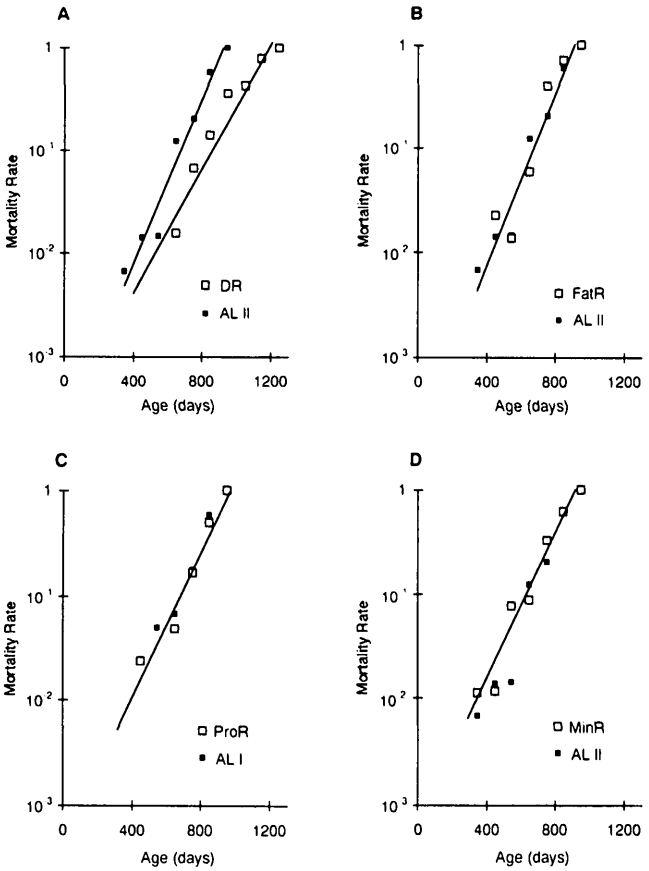

Figure 2. The age-specific mortality rates of neoplastic disease. The lines are least-squares fittings to the logarithm of mortality rates. The least-squares estimates of only Group FatR, ProR, and MinR are shown in parts B, C, and D respectively.

Table 3. Onset Rates of Leukemia Relative to Relevant Control Groups AL I and AL II

Dietary Group	Fatal Leukemia			Incidental Leukemia			Combined			
	O	E	O/E	O	E	O/E	O	E	O/E	*p*-value
FatR	16	12.74	1.26	18	16.61	1.08	34	29.35	1.16	.204
ProR	3	3.23	0.93	5	4.49	1.11	8	7.72	1.04	.858
MinR	11	10	1.1	6	11.48	0.52	17	21.48	0.79	.072
DR	30	35.72	0.84	9	13.21	0.68	39	48.93	0.80	.002
AL I	2	—	—	3	—	—	5	—	—	—
AL II	13	—	—	25	—	—	38	—	—	—

Notes. O: Observed number of rats bearing leukemia; E: Expected number of rats bearing leukemia; O/E: Relative onset rate.
p-values: For comparison to AL I or II.

Table 4. Onset Rates of Pituitary Adenoma Relative to Relevant Control Groups AL I and AL II

Dietary Group	Fatal Pituitary Adenoma			Incidental Pituitary Adenoma			Combined			
	O	E	O/E	O	E	O/E	O	E	O/E	*p*-value
FatR	2	2.00	1.00	12	14.63	0.82	14	16.63	0.84	.315
ProR	3	3.81	0.79	2	3.33	0.60	5	7.14	0.70	.183
MinR	5	3.42	1.46	12	13.80	0.87	17	17.22	0.99	.936
DR	3	4.63	0.65	4	12.64	0.32	7	17.27	0.41	<.0001
AL I	3	—	—	5	—	—	8	—	—	—
AL II	3	—	—	22	—	—	25	—	—	—

Notes. O: Observed number of rats bearing pituitary adenoma; E: Expected number of rats bearing pituitary adenoma; O/E: Relative onset rate.
p-values: For comparison to AL I or II.

which is used as a descriptive index of the effect of a dietary modulation on the occurrence of these neoplasms. In this report, the O/E ratio is referred to as the relative onset rate for convenience in accord with the usage of Peto et al. (1980). The DR group had an approximately 20% lower onset rate of leukemia than Group AL II ($p < .01$). The MinR group also appeared to have about a 20% lower onset rate of leukemia than Group AL II but the statistical significance is marginal ($p < .08$). The onset rate for leukemia for the FatR and ProR Groups was not less than those of the relevant control groups.

Group DR had an onset rate of pituitary adenoma that was about 60% less than Group AL II ($p < .01$). The relative onset rate of pituitary adenoma appeared to be reduced in FatR and ProR groups but the difference was not statistically significant. Group MinR did not differ from Group AL II in regard to onset rate of pituitary adenoma.

DISCUSSION

The conditional survival curves and mortality rates of fatal neoplasms indicate that only the dietary regimen which included energy restriction delayed the occurrence of death due to neoplastic diseases. Individual component restrictions without energy restriction did not significantly delay death due to neoplasm. In other words, in the male Fischer 344 rat only dietary restriction which included energy restriction increased longevity by influencing neoplastic disease. The increase in longevity in rats that were fed the protein-restricted but not the energy-restricted diet (Yu et al., 1985) was not due to the retardation of neoplastic disease processes.

However, analyses of relative onset rates of leukemia and pituitary adenoma suggest that individual component restrictions may influence certain types of neoplasms. Protein and fat restriction tended to delay the occurrence of pituitary adenoma but not leukemia. In contrast, mineral restriction delayed the occurrence of leukemia, but not pituitary adenoma. Since the survival curves of the ad libitum fed groups (FatR, ProR, MinR) in Figure 1 were similar to those of the control groups, it appears that the influence on longevity of delaying the occurrence of one type of neoplasm must be offset by the occurrence of another fatal neoplasm. Only dietary restriction involving energy restriction was found to broadly modulate the onset or progression of neoplastic lesions and thereby delay death due to neoplasms resulting in an increased longevity. Our findings on spontaneous neoplasms are similar to those reported by Ross and Bras (1965) and Carroll and Khor (1975); protein or fat restriction without energy in diet does not broadly modulate the occurrence of neoplasms.

The validity of the analyses in this study depends upon the accuracy of the classification of neoplastic lesions and the independence of the neoplastic and non-neoplastic causes of death in rats. The accuracy of the classification can always be criticized. However, the profound bias due to intercurrent mortality in data derived from rats dying spontaneously makes it necessary to address this issue. For example, the findings of a lower prevalence or the later appearance of neoplastic lesions in relation to diet in rats dying spontaneously may contain this bias. The method of Peto et al. (1980) is useful in attempting to correct for this bias when using data obtained at the time of death. In this way, information is

gained on how neoplastic lesions or non-neoplastic lesions influence the life span and aging processes. However, success in this endeavor requires reliable information on cause of death.

In our analysis, the assumption is made that rats in the control groups (AL I and AL II) and the other groups (FatR, ProR, MinR, and DR) are all equally likely to die from a neoplastic lesion at any particular stage in its development. Of course, this assumption may not always be reasonable. For example, rats under a stress like that of renal insufficiency might die more rapidly than otherwise healthy rats once they develop a neoplastic lesion. In this study, conditional survival curves and mortality rates were generated from rats bearing probably fatal or fatal neoplasms without considering the contribution of non-neoplastic lesions to death. Therefore, death due to neoplastic lesions may be overestimated, particularly in Group AL II as compared to Group DR. Thus, the actual difference in the survival distribution or mortality rates from neoplasms between Group AL and DR may be somewhat smaller than the difference estimated in this study.

It seems likely that energy restriction is the major factor in the retardation of neoplastic disease by restriction of food intake. However, a systematic investigation of the effects of the restriction of the carbohydrate and fiber components remains to be done. The vitamin content in the group DR diet was adjusted so that the absolute intake of the DR rats was the same as the ad libitum fed rats. However, simple dietary restriction in which all the components were reduced by the same amount also has been shown to retard neoplastic diseases and to increase longevity (Tannenbaum et al., 1945; Tucker, 1979). It is, of course, possible but doubtful that synergistic interactions between restricted components might be a major factor in the action of dietary restriction.

Mechanisms by which the restriction of energy intake modulates the occurrence of neoplasms still remain to be elucidated. Free radicals and oxidative stress have been colinked to the aging processes and to carcinogenesis (Harman, 1984). Dietary restriction has been shown to modulate cellular damage mediated by active oxygen species (Laganiere and Yu, 1989a, 1989b; Weindruch, 1989) and in this way could retard neoplastic disease.

However, there are many other possible mechanisms by which the restriction of energy intake may influence neoplastic disease. Indeed, if such restriction reduces the number of a particular type of cell, e.g., large granular splenic lymphocytes in the case of mononuclear cell leukemia of Fischer 344 rats (Moloney and King, 1973), the reduced number of cells available for transformation may solely account for the delay in occurrence of the neoplasm. Unfortunately, the information needed to assess this and many other possibilities is currently lacking.

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Address correspondence to Dr. Byung Pal Yu, Department of Physiology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284-7756.

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