Aging Clin. Exp. Res. 8: 254-262, 1996

Influence of dietary components on occurrence of and mortality due to neoplasms in male F344 rats

I. Shimokawa[1], Y. Higami[1], B.P. Yu[2], E.J. Masoro[2], and T. Ikeda[1]

[1]Department of Pathology, Nagasaki University School of Medicine, Nagasaki City, Japan, [2]Department of Physiology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas, U.S.A.

ABSTRACT. The influence of dietary components on the occurrence of, and mortality from spontaneous neoplasms in male F344 rats was investigated. The dietary regimens studied included restriction of specific dietary components (energy, fat, protein and mineral), as well as different sources of dietary protein (casein, soy protein and lactalbumin). A statistical approach based on contributing causes of death was used to obtain the mortality due to all neoplasms, and the relative onset rate of frequently observed neoplasms, e.g., leukemia, pituitary adenoma, testicular interstitial cell tumor, etc. Only the regimen involving energy restriction reduced the mortality due to all neoplasms. Neither reduction of individual components without energy restriction, nor replacement of casein with soy protein or lactalbumin as the protein source affected mortality. Analyses of the relative onset rate of selected neoplasms also indicated that only a reduction of energy intake suppressed the occurrence of most of these neoplasms. Other dietary regimens, at most, suppressed a few types of neoplasm. It is concluded that a reduction in energy intake is a key dietary factor for the prevention of neoplastic diseases in rats.

(Aging Clin. Exp. Res. 8: 254-262, 1996)

INTRODUCTION

It is generally accepted that dietary or nutritional factors modulate the development of cancer with increasing age. A comprehensive review of this subject performed in the U.S.A. in 1982 (1) suggested that a high fat diet, and probably a high protein diet, could increase the risk of certain cancers in humans and experimental animals. Dietary restriction (or food restriction) in rodents has long been known to reduce the incidence of neoplasms (2), but the role of the reduction of energy intake in this inhibition of neoplastic disease was inconclusive because of the potential confounding influence of reducing intake of dietary fat, and the other dietary constituents. However, several reports have indicated an independent role for the reduction in the dietary energy intake in the inhibition of neoplasia (3-6).

At the University of Texas Health Science Center at San Antonio (UTHSCSA), life-span studies in male F344 rats have been carried out under various dietary regimens to evaluate the role of specific nutrients under the anti-aging action of dietary restriction (7-12). These studies have yielded data which are suitable for the analysis of effects of dietary components on neoplasia, because the semisynthetic base diet used enabled restriction or replacement of dietary components without altering energy intake.

In the present report, the influence of reduction of dietary energy, fat, mineral and protein intake, and of dietary protein source on neoplastic disease was assessed. This assessment involved the pathological analysis of spontaneously occurring neoplasms in relation to occurrence, spontaneous death and life span.

Biases occur in the prevalence data of neoplasms found in spontaneously dying rats, when overall mortality characteristics differ between experimental groups. For example, if one group of rats died from a non-neoplastic disease prematurely, the prevalence of a particular type of neoplasm would appear to be lower than in another group of rats that live longer, even if underlying age-specific onset rates are equal in the two groups. In fact, in the study of Maeda et al. (8) carried out at UTHSCSA, the proportion of

Key words: Diet, dietary restriction, neoplasm.

Correspondence: B.P. Yu, Ph.D., Department of Physiology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7756, U.S.A.

Received July 24, 1995; accepted in revised form March 29, 1996.

rats bearing neoplasms during life time was significantly larger in dietary restricted (DR) rats than in control rats fed *ad libitum* (AL); 67.9% in DR rats and 53.5% in AL rats. In that study, it was shown that dietary restriction markedly suppresses nephropathy and cardiomyopathy, potentially lethal disorders in AL male rats, and increases life span. Moreover, because dietary restriction did not suppress neoplastic diseases as much as nephropathy and cardiomyopathy, life time prevalence of neoplasms was higher in DR rats at the time of spontaneous death. Peto et al. (13) and Gart et al. (14) have addressed the biases inherent in long-term animal studies, and have described a way to circumvent the problem. In the present report, this statistical approach was utilized. Specific aspects of these findings have previously been published (15-18).

MATERIALS AND METHODS

Source of data

The data used in this analysis were obtained from the studies conducted at UTHSCSA from 1979 to 1991 which explored effects of reduction of dietary energy (10, 11), protein (8), fat (10) and mineral (10), and of replacement of casein with soy protein (9) and lactalbumin (12).

Rat maintenance and dietary manipulations

Rat maintenance and dietary manipulations were described in previous papers (7-12). Briefly, specific pathogen-free male F344 rats were purchased as weanlings (aged 26-30 days) from the Charles River Laboratories at Kingston, N.Y. The rats were cared for, and used in accordance with the guidelines of UTHSCSA.

Until 6 weeks of age, all rats were fed a semisynthetic diet (Diet A) *ad libitum* containing casein as the protein source; this was the standard diet used at UTHSCSA. The control rats continued to receive the diet *ad libitum* throughout life. The control groups relevant to respective experimental groups are desig-

Table 1 - *Composition of diets.*

Component (gram/100 g of diets)	Diet A	Diet B	Diet C	Diet E	Diet F	Diet S	Diet L
Casein	21	21	12.6	21	21	0	0
Soy protein	0	0	0	0	0	21	0
Lactalbumin	0	0	0	0	0	0	21
D,L Methionine	0.15	0.15	0.09	0.15	0.15	0.35	0.15
Dextrin	43.65	39.55	52.11	47.65	45.65	43.45	43.65
Sucrose	15	15	15	15	15	15	15
Corn oil	10	10	10	6	10	10	10
Ralston-Purina vitamin mix#	2	3.33	2	2	2	2	2
Choline chloride	0.2	0.33	0.2	0.2	0.2	0.2	0.2
Ralston-Purina mineral mix#	5	7.64	5	5	3	0	0
Adjusted Ralston-Purina mineral mix**	0	0	0	0	0	5	5
Fiber	3	3	3	3	3	3	3
Physiologic caloric value (kcal/g)*	4.10	4.04	4.10	3.97	4.18	4.10	4.10

Diet A: Semisynthetic diet for control groups, Group AL-I, Group AL-II, Group AL (cas)-II, Group AL (cas)-III.
Diet B: Diet for restriction of dietary (energy) intake (Group DR).
Diet C: Diet for restriction of protein intake (Group ProR).
Diet E: Diet for restriction of fat intake (Group FatR).
Diet F: Diet for restriction of mineral intake (Group MinR).
Diet S: Diet for replacement of casein with soy protein [Group AL (soy)].
Diet L: Diet for replacement of casein with lactalbumin [Group AL (lact)].

The details of the composition of the Ralston-Purina vitamin mix and the Ralston-Purina mineral mix were reported previously (45).

**The Adjusted Ralston-Purina mineral mix refers to a preparation that was modified so that Diet S and L had the same mineral content (including phosphorus content) as Diet A.

* O_2 consumption was measured for a group of *ad libitum* fed (Diet A) and dietary restricted rats (Diet B) and both groups had the same O_2 consumption per unit of lean body mass (46).

nated as Group AL-I, AL-II, AL (cas)-II, and AL (cas)-III in the present paper. The experimental groups were switched to their respective diets at 6 weeks of age (see Table 1 for diet compositions). Rats in Group DR were fed Diet B at approximately 60% of the mean energy intake of Group AL-II. Group ProR was fed *ad libitum* Diet C which reduced protein (casein) intake to 60% of that in Group AL-I. Groups FatR and MinR were fed *ad libitum* Diets E and F, which reduced the intake of fat or mineral respectively to 60% of that in Group AL-II. Group AL (soy) and AL (lact) were fed *ad libitum* Diets G and H which contain soy protein and lactalbumin, respectively, as the protein source instead of casein.

The mean food intake per rat (kcal per day), body weight and the growth pattern were monitored during the studies (7-12). Except for Group DR, these parameters were very similar between each experimental group and its relevant control group. Rats in Group DR weighed less than the control rats (Group AL-II) for most of the life span.

Longevity characteristics have been presented in previous papers (7-12). The median length of life, and the age of the 10th percentile survivors were significantly greater in Groups DR, ProR and AL (soy), as compared to the appropriate control groups (8, 9, 11), while those of Groups FatR, MinR and AL (lact) did not differ from those of the respective control groups (10, 12).

Procedures for pathological examination

Procedures for pathological examination of spontaneously dying rats have been described in previous papers (8, 9). Briefly, almost all the major organs of spontaneously dying rats, and any other organ or tissue with gross lesions, were examined histologically. It should be emphasized that the diagnosis of mononuclear cell leukemia was revised by reviewing microscopic slides without referring to previous diagnoses, according to the revised diagnostic criteria for this type of leukemia described previously (15). A diagnostic category in the present study, subcutaneous mesenchymal tumor, included the several types of mesodermal neoplasms listed in the atlas of "Pathology of the Fischer 344 Rat" (19). This diagnostic category may also include a few cases of mammary fibroadenoma, in which fibrous components markedly proliferated. As mentioned by Elwell et al. (19), routine histopathologic examination with H.E. sections did not always reveal diagnostic hallmarks of specific types of mesodermal tumors; therefore, this group of tumors was collectively referred to as subcutaneous mesenchymal tumors.

In order to calculate mortality due to all neoplasms and the relative onset rate, a pathologist (I.S.) reviewed autopsy records and histopathological specimens from previous studies, following the method of Peto et al. (13), without reference to the diet group, or age of rats. All the neoplasms observed in each rat at autopsy were scored with regard to the likelihood of cause of death in rats as follows: [1] incidental; [2] probably incidental; [3] probably fatal; [4], fatal. A neoplasm that either directly or indirectly killed a rat was given a score of [3] or [4], and was classified as fatal. Neoplasms considered not to be involved in death were given a score of [1] or [2], and designated incidental.

Peto's method is based on the assumption that the cause of death can be determined for each rat. Clearly, incorrect information regarding cause of death can create substantial biases (20). Although it is difficult to verify the validity of the basic assumption of Peto's method, attempts to do so for selected neoplasms, leukemia, pituitary adenoma and pheochromocytoma were carried out, and presented in previous reports. These assessments involved comparing the prevalence of a neoplasm in categories [1] and [2] in rats that died spontaneously with the prevalence of that neoplasm in sacrificed rats (16, 18). These prevalences should not significantly differ if the basic assumption is correct, provided that spontaneous death from causes other than that neoplasm is under the same censorship as that of the randomly sacrificed rats. The results of this analysis are consistent with the validity of the basic assumption of Peto's method.

Statistical analysis

The conditional mortality function for all neoplasms was generated using a nonparametric product limit estimator (21). In this study, $Q^{\bullet}(t)$ is formally defined as the probability that a rat will die before time t of a neoplastic disease, given that it does not die of non-neoplastic diseases before t. $Q^{\bullet}(t)$, which is a product limit estimator, was calculated by the following formula:

$$Q^{\bullet}(t)=1-\prod_{t_i\leq t}\frac{n_i-d_i}{n_i}$$

where n_i is the number of rats alive just before t_i, and d_i is the number of rats dying of neoplasms at time t_i. The mortality distributions were compared by the Gehan-Wilcoxon test (22).

The relative onset rate as defined by Peto et al. (13) was calculated for leukemia, pituitary adenoma, thyroidal C-cell tumor, adrenal pheochromocytoma, pancreatic islet cell tumor, testicular interstitial cell tumor, and subcutaneous mesenchymal tumor, all of which are common neoplasms in male F344 rats. The relative

onset rate of a neoplasm is actually a ratio of the total observed number of rats with the neoplasm, and the total expected number of rats with the neoplasm. The relative onset rate is a descriptive index useful in determining whether or not a dietary modulation influences the occurrence of a neoplasm. The expected number of rats with the neoplasm was calculated by analyzing the death rate and the prevalence rate separately.

The death rate analysis involves the following equation:

$$Ed_k = m_k \bullet (y_k + x_k)/(m_k + n_k)$$

where Ed_k is the expected number of rats in an experimental group with the neoplasm considered as fatal, i.e., scored in [3] or [4], during a day k; y_k is the observed number of rats in an experimental group with the fatal neoplasm during a day k; x_k is the observed number of rats in a relevant control group with the fatal neoplasm during a day k; m_k is the number of rats in an experimental group surviving at the beginning of a day k; and n_k is the number of rats in a relevant control group surviving at the beginning of a day k. Rats with the neoplasm scored in an incidental context, [1] or [2], were treated like all other rats that die of causes other than the neoplasm.

The prevalence analysis involves the following equation:

$$Ep_i = m_i \bullet (y_i + x_i)/(m_i + n_i)$$

where Ep_i is the expected number of rats in an experimental group with the neoplasm considered as incidental, i.e., scored in [1] or [2], during a time interval i; y_i is the observed number of rats in an experimental group with the incidental neoplasm during time interval i; x_i is the observed number of rats in a relevant control group with the incidental neoplasm during time interval i; m_i is the number of dying rats in an experimental group during time interval i; and n_i is the number of dying rats in a relevant control group during time interval i. It should be noted that rats which died from the neoplasm under analysis were excluded from m_i and n_i.

The summarized onset rate ("relative onset rate") is assessed by the following equation:

$$O/E = (\Sigma\, y_k + \Sigma\, y_i)/(\Sigma\, Ed_k + \Sigma\, Ep_i).$$

These procedures eliminate the bias due to intercurrent mortality as effectively as is possible based on the available data. The method for calculation, and the χ^2 test for difference in incidence of the neoplasm between an experimental and a control group has been described in detail by Peto et al. (13). In the present study, many comparisons in incidence between two groups were made with statistical significance being accepted at $p<0.05$; therefore, the probability of drawing an incorrect conclusion for the relative onset rate increased.

RESULTS

The number of rats studied, the number that died spontaneously and the number and percent that died from neoplasms are presented in Table 2. The number of rats bearing common types of neoplasm is

Table 2 - *Number of rats dying spontaneously from neoplasms.*

	Dietary Group									
	DR	FatR	MinR	AL-II	ProR	AL-I	AL (soy)	AL (cas)-II	AL (lact)	AL (cas)-III
No. of rats studied	153	92	82	155	117	120	60	60	40	40
Spontaneous deaths	89	71	69	111	69	71	60	60	40	40
Death due to neoplasm	41	30	32	32	24	16	39	14	26	19
(%)#	(46.1)	(42.3)	(46.4)	(28.8)	(34.8)	(22.5)	(65.0)	(23.3)	(65.0)	(47.5)
Mean Maximum Body Weight (g)	328.7	523.9	532.9	510.9	578.5	560.4	522.8	510.9	527.2	540.8
Average Food Intake/day (g)	7.9	13.7	12.8	12.8	13.7	12.8	13.2	13.3	12.8	13.1
Life span (weeks)										
Median	133	109	109	104	115	100	120	104	110	110
Maximum	182	137	134	141	138	134	146	141	144	154
Literature Reference	11	10	10	10, 11	7, 8	7, 8	9	9	12	12

#Proportion of deaths due to neoplasm in total spontaneous deaths in parenthesis.
AL-II is the control group for Group DR, FatR and MinR. AL-I is the control group for Group ProR.
AL (cas)-II and AL (cas)-III are the control groups for Group AL (soy) and AL (lact) respectively.

Table 3 - *Number of rats bearing selected neoplasms.*

	Dietary Group									
	DR	FatR	MinR	AL-II	ProR	AL-I	AL (soy)	AL (cas)-II	AL (lact)	AL (cas)-III
No. of rats examined	89	71	69	111	69	71	60	60	40	40
Leukemia	(43.8)	(47.9)	(24.6)	(34.2)	(11.6)	(7.0)	(58.3)	(46.7)	(37.5)	(20.0)
fatal	30	16	11	13	3	2	16	8	9	3
incidental	9	18	6	25	5	3	19	20	6	5
Pituitary adenoma	(7.9)	(19.7)	(24.6)	(22.5)	(7.2)	(11.3)	(18.3)	(26.7)	(57.3)	(50.0)
fatal	3	2	5	3	3	3	4	1	10	7
incidental	4	12	12	22	2	5	7	15	13	13
Thyroidal C-cell tumor	(7.9)	(21.1)	(8.7)	(15.3)	(5.8)	(7.0)	(18.3)	(11.7)	(5.0)	(7.5)
fatal	1	0	0	2	0	0	0	1	0	0
incidental	6	15	6	15	4	5	11	6	2	3
Adrenal pheochromocytoma	(5.6)	(5.6)	(15.9)	(7.2)	(13.0)	(8.5)	(16.7)	(10.0)	(10.0)	(10.0)
fatal	2	0	4	0	2	0	1	13	1	0
incidental	3	4	7	8	7	6	9	(5.0)	3	4
Pancreatic islet cell tumor	(13.5)	(14.1)	(11.6)	(10.8)	(7.2)	(7.0)	(21.7)	(10.0)	(10.0)	(10.0)
fatal	0	0	0	0	0	0	0	1	0	0
incidental	12	10	8	12	5	5	13	6	3	4
Testicular interstitial cell tumor	(49.4)	(74.6)	(82.6)	(74.8)	(60.9)	(83.1)	(73.3)	(80.0)	(50.0)	(77.5)
fatal	0	0	0	0	0	0	0	0	0	0
incidental	44	53	57	83	42	59	50	48	20	31
Subcutaneous mesenchymal tumor	(10.1)	(22.5)	(21.7)	(7.2)	(20.5)	(1.4)	(31.7)	(3.3)	(10.0)	(17.5)
fatal	4	5	4	5	5	0	10	2	3	1
incidental	5	11	11	3	3	1	9	0	1	6

Prevalence of each type of neoplasm in parenthesis. fatal: No. of rats bearing the type of neoplasm considered fatal or probably fatal; incidental: No. of rats bearing the type of neoplasm considered incidental or probably incidental. The categorization of a neoplasm into fatal or incidental context was done, following the method of Peto et al. (13). AL-II is the control group for Group DR, FatR and MinR. AL-I
is the control group for Group ProR. AL (cas)-II and AL (cas)-III are the control groups for Group AL (soy) and AL (lact) respectively.

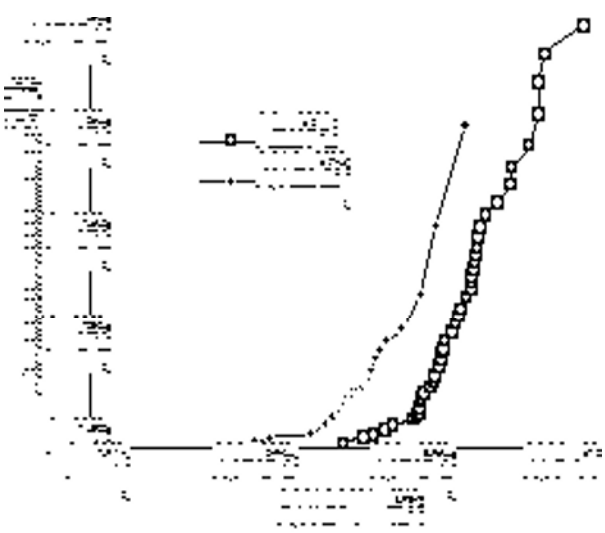

Figure 1 - *Cumulative mortality of all neoplasms in rats fed a semisynthetic diet (Group AL-II) and rats (Group DR) fed 60% of the mean dietary and energy intake of the rats fed* ad libitum

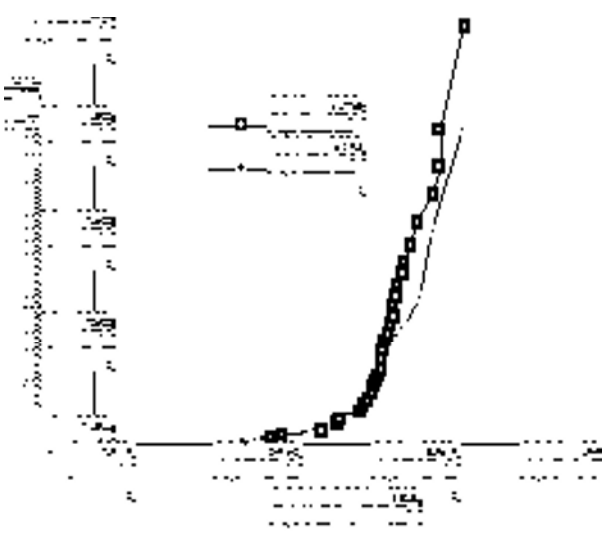

Figure 2 - *Cumulative mortality of all neoplasms in rats fed a semisynthetic diet (Group AL-II) and rats (Group FatR) fed a diet resulting in a reduced fat intake without a reduction in energy intake.*

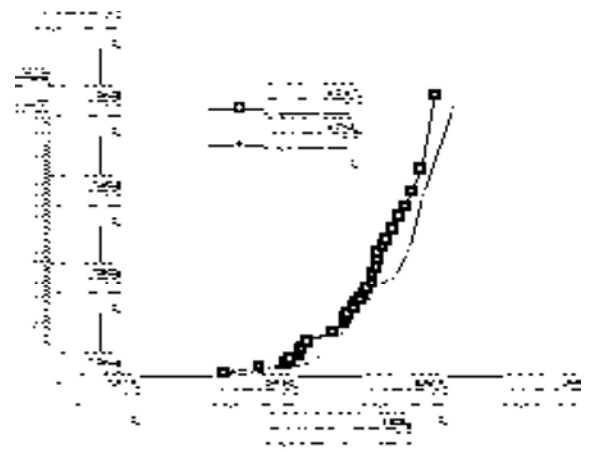

Figure 3 - *Cumulative mortality of all neoplasms in rats fed a semisynthetic diet (Group AL-II) and rats (Group MinR) fed a diet resulting in a reduced mineral intake without a reduction in energy intake.*

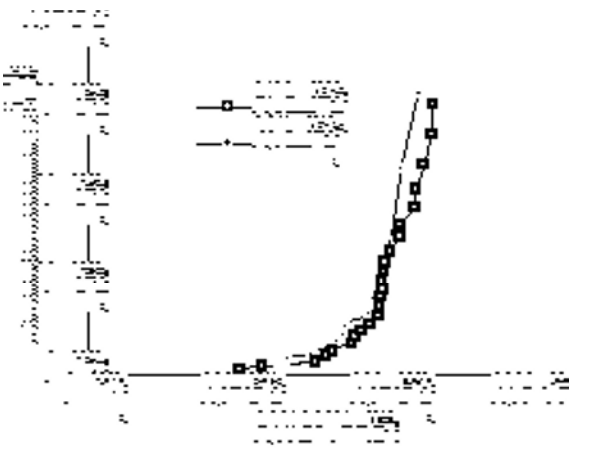

Figure 4 - *Cumulative mortality of all neoplasms in rats fed a semisynthetic diet (Group AL-I) and rats (Group ProR) fed a diet resulting in a reduced protein intake without a reduction in energy intake.*

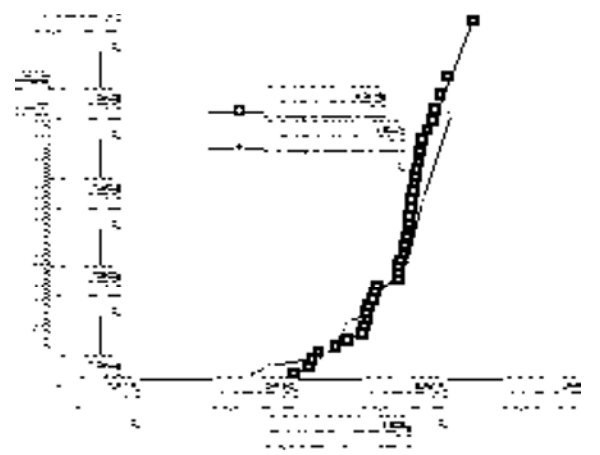

Figure 5 - *Cumulative mortality of all neoplasms in rats fed a semisynthetic diet (Group AL (cas)-II) and rats (Group (soy)) fed a diet replacing casein with soy protein as the protein source.*

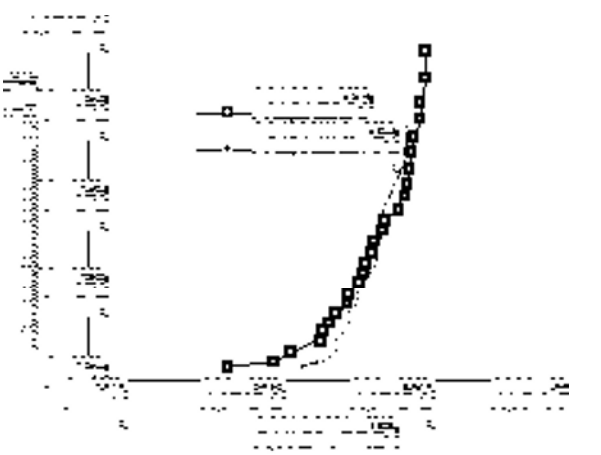

Figure 6 - *Cumulative mortality of all neoplasms in rats fed a semisynthetic diet (Group AL (cas)-III) and rats (Group (lact)) fed a diet replacing casein with lactalbumin as the protein source.*

summarized in Table 3.

Conditional mortality curves for neoplasms

Only in Group DR did the mortality curve for neoplasms differ from that in the control group ($p<0.01$). The age at death due to neoplasms seemed to be delayed by 150 to 200 days in Group DR (Fig. 1). The curves of the other diet groups did not differ significantly from those in the respective control curves (Figs. 2-6).

Relative onset rates of neoplasms

In Group DR, most of the neoplasms examined occurred less frequently than expected ($p<0.01$, Table 4), although no significant influence was observed in the occurrence of islet cell tumors of the pancreas or subcutaneous tumors. In Group FatR, no significant suppressive effect was observed in the neoplasms examined; on the contrary, subcutaneous tumors occurred more frequently than expected ($p<0.05$). In Group MinR, the occurrence of thyroidal C-cell tumors was significantly reduced ($p<0.05$); leukemia

Table 4 - *Relative onset rate of selected neoplasms.*

Type of neoplasm	Dietary Group					
	DR	FatR	MinR	ProR	Soy	Lact
Leukemia	0.80**	1.16	0.79	1.04	1.07	0.98
Pituitary adenoma	0.41**	0.84	0.99	0.70	0.83	1.06
Thyroidal C-cell tumor	0.56**	1.07	0.62*	1.08	1.07	0.83
Adrenal pheochromocytoma	0.55**	0.70	1.31	1.39	1.03	1.16
Pancreatic islet cell tumor	0.84	1.01	1.03	0.99	1.15	1.16
Testicular interstitial cell tumor	0.72**	0.98	1.01	0.91**	0.99	0.83*
Subcutaneous mesenchymal tumor	0.97	1.57**	1.62*	1.22	1.45*	0.65

Each value represents summarized onset rate of neoplasm relative to relevant control groups.
Statistical significance: *$p<0.05$, ** $p<0.01$.

might be suppressed ($0.05 < p<0.10$), while subcutaneous tumors occurred more frequently ($p<0.05$), but other neoplasms were not affected. In Group ProR, only the occurrence of testicular interstitial cell tumors was significantly reduced ($p<0.05$). In Group AL (soy), subcutaneous tumors occurred more often than expected ($p<0.05$), and other neoplasms were not influenced. In Group AL (lact), the occurrence of testicular interstitial cell tumors was reduced ($p<0.05$), but no significant influence was observed in the occurrence of the other types of neoplasms.

DISCUSSION

Adjustment for intercurrent mortality is an important process in long-term assessment of neoplasia (13, 14). Dietary restriction, protein restriction and soy protein diet significantly retarded the development of nephropathy, which is a common cause of intercurrent mortality (8, 9). This dietary effect on intercurrent mortality probably was in part responsible for the considerable differences between diets in the proportion of deaths due to neoplasms, and in the lifetime prevalence of some neoplasms. In the present study, the problem of intercurrent mortality was circumvented by classifying neoplasms based on the likelihood of cause of death in each rat. The method of Peto et al. (13) was the practical approach used to solve this issue, although this method is difficult to justify rigorously (14).

Monitoring the amount of dietary intake and the growth pattern of rats is important in this type of analysis, because a particular dietary regimen may influence the occurrence of neoplasms by altering dietary energy intake, which confounds assessing the effects of a specific dietary component. Our dietary manipulations were precisely controlled, and there was no significant difference in energy intake between dietary groups other than the dietary-restricted group.

The present study indicated that only the dietary regimen involving a reduction in energy intake reduces the mortality due to all neoplastic diseases. Restriction of fat, mineral or protein without a reduction of energy intake, or replacement of casein with soy protein or lactalbumin as the protein source, had no significant effect on mortality due to neoplasia. Analyses of the relative onset rate of the frequently observed neoplasms provided further insight. Only the restriction of dietary energy intake suppressed a wide range of neoplasms, and suppressed mortality due to neoplasms in general. The dietary regimens without a reduction of energy intake in some instances reduced the occurrence of a specific type of neoplasm, but also resulted in the more frequent occurrence of other types of neoplasms than expected.

A similar effect of dietary protein has been reported for spontaneous neoplasms in male Sprague-Dawley rats (23). That study indicated that an isocaloric, but low protein intake decreased the risk of neoplasms in the pituitary gland, thyroid gland and pancreas, but the risk of lymphoreticular and hematopoietic neoplasms was increased. A comprehensive study conducted by Roe et al. (24) also revealed that reduction of energy intake caused marked suppression of spontaneous tumorigenesis in Wistar rats, although relatively low protein diet did to some extent decrease the incidence of tumors in most sites. In a study by Carrol and Khor (25), evidence was presented that a low-fat diet might decrease the risk of certain, but not all types of neoplasms, independent of dietary energy intake.

Most studies on diet and cancer have focused specific types of cancer, and have stressed the impor-

tance of a specific dietary constituent (26-28). However, a variety of neoplastic diseases occur competitively in animals as well as humans, and there is little evidence that modulation of a specific nutrient can reduce the risk of a broad spectrum of neoplastic disease. If our research with rats can be extrapolated to humans, it appears that the best way to prevent cancer in people may be simply to reduce total dietary energy intake in a fashion which avoids malnutrition. Indeed, Lutz and Schlatter (29) estimated by epidemiological and experimental data that three-quarters of cancer cases attributed to dietary factors in Switzerland (60 000 cancer cases per one million lives) might be due to overnutrition.

The molecular mechanisms of carcinogenesis and the modulation of its progression are quite complex, and remain to be elucidated. However, substantial evidence implicates the involvement of free radicals and other reactive pro-oxidants in many of the stages of carcinogenesis. Multi-step processes comprised of a sequence of changes in DNA, such as mutations which activate proto-oncogens or inactivate tumor suppressor genes, as well as gross chromosomal aberrations are involved in carcinogenesis (30, 31). These genetic changes are induced by many endogenous and exogenous factors. Of these factors, reactions of free radicals or reducing sugars and subsequent Maillard reaction with macromolecules in the cell may be of particular importance (32-34). Recently, Kristal and Yu (35) proposed a new hypothesis of aging based on a premise that the age-related deterioration and damage of cellular constituents including DNA are exacerbated by the sum of the synergestic interactions induced by free radicals, glycation, and Maillard reactions. In this regard, it is important to recognize that recently dietary restriction has been reported to exhibit potent and wide-ranging effects on antioxidant defenses and free radical metabolism (36-38), and reduce glycation products (39). A possible mechanism by which dietary restriction suppresses neoplasms may relate to its ability to reduce the amount of DNA damage, and the subsequent DNA mutations which usually occur during aging. Another potentially important factor that is modulated by dietary restriction is DNA repair capacity (40), which if up-regulated also would be expected to reduce the level of DNA damage. These findings are further supported by the evidence of the protective action of dietary restriction against the oxidative damage of DNA (41).

In addition, we emphasize the importance of dietary restriction in decreasing cell number and proliferation. Genetic changes tend to occur during mitosis (42). A decreased rate of cell division by dietary restriction could minimize the risk of the genetic changes that are potentially associated with the occurrence of neoplasms. Sinha et al. (43) suggested that DR suppressed chemically induced tumorigenesis in the mammary gland by slowing the cell division in the mammary epithelium when the carcinogen is administered to rats. Lagopoulos et al. (44) also proposed the possibility that dietary restriction decreased the proliferation of hepatocytes by reducing serum insulin level, a mitogen for hepatocytes, and inhibited chemically induced hepatic tumors. The smaller number of cells available for neoplastic transformation may account for the decrease in occurrence of neoplasms. How dietary energy restriction modulates cell proliferation should be the subject of intensive study.

There are many other ways by which dietary restriction could suppress carcinogenesis. Further research focused on molecular changes induced by the restriction of dietary energy intake is needed.

ACKNOWLEDGEMENT

This research was supported by USPHS grant number AG-01188.

REFERENCES

1. *Diet, Nutrition and Cancer.* National Academy of Sciences, Washington, D.C., 1982.
2. Weindruch R., Walford R.L.: *The Retardation of Aging and Disease by Dietary Restriction*. Charles C. Thomas, Springfield, IL, 1987.
3. Kritchevsky D., Weber M.M., Klurfeld D.M.: Dietary fat versus caloric content in initiation and promotion of 7,12-dimethylbenz(a)anthracene-induced mammary tumorigenesis in rats. *Cancer Res.* 44: 3174-3177, 1984.
4. Boissoneault G.A., Elson C.E., Pariza M.W.: Net energy effects of dietary fat on chemically-induced mammary carcinogenesis in F344 rats. *J. Natl. Cancer Inst.* 76: 335-338, 1986.
5. Albanes D.: Total calories, body weight, and tumor incidence in mice. *Cancer Res.* 47: 1987-1992, 1987.
6. Clinton S.K., Imrey P.B., Mangian H.J., Nandkumar S., Visek W.J.: The combined effects of dietary fat, protein, and energy intake on azoxymethane-induced intestinal and renal carcinogenesis. *Cancer Res.* 52: 857-865, 1992.
7. Yu B.P., Masoro E.J., McMahan C.A.: Nutritional influences on aging of Fischer 344 rats: I. Physical, metabolic, and longevity characteristics. *J. Gerontol.* 40: B657-B670, 1985.
8. Maeda H., Gleiser C.A., Masoro E.J., Murata I., McMahan C.A., Yu B.P.: Nutritional influences on aging of Fischer 344 rats: II. Pathology. *J. Gerontol.* 40: B671-B688, 1985.
9. Iwasaki K., Gleiser C.A., Masoro E.J., McMahan C.A., Seo E.J., Yu B.P.: The influence of dietary protein source on longevity and age-related disease processes of Fischer rats. *J. Gerontol.* 43: B5-B12, 1988.
10. Iwasaki K., Gleiser C.A., Masoro E.J., McMahan C.A., Seo

E.J., Yu B.P.: Influence of the restriction of individual dietary components on longevity and age-related disease of Fischer rats: The fat component and the mineral component. *J. Gerontol.* 43: B13-B21, 1988.

11. Masoro E.J., Iwasaki K., Gleiser C.A., McMahan C.A., Seo E.J., Yu B.P.: Dietary modulation of the progression of nephropathy in aging rats: an evaluation of the importance of protein. *Am. J. Clin. Nutr.* 49: 1217-1227, 1989.
12. Shimokawa I., Higami Y., Hubbard G.B., McMahan C.A., Masoro E.J., Yu B.P.: Diet and suitability of the male Fischer 344 rat as a model for aging research. *J. Gerontol.* 48: B27-B32, 1993.
13. Peto R., Pike M.C., Day N.E., Gray R.G., Lee P.N., Parish S., Peto J., Richards S., Wahrendorf J.: Long-term and short-term screening assays for carcinogens: a critical appraisal. IARC Monograph (Suppl. 2), 1980.
14. Gart J.J., Krewski D., Lee P.N., Tarone R.E., Wahrendorf J.: *Statistical methods in cancer research. Vol. III-The design and analysis of long-term animal experiments*. Oxford University Press, Oxford, UK, 1986.
15. Shimokawa I., Yu B.P., Masoro E.J.: Influence of diet on fatal neoplastic disease in male Fischer 344 rats. *J. Gerontol.* 46: B228-B232, 1991.
16. Shimokawa I., Yu B.P., Higami Y., Ikeda T., Masoro E.J.: Dietary restriction retards onset but not progression of leukemia in male F344 rats. *J. Gerontol.* 48: B68-B73, 1993.
17. Higami Y., Yu B.P., Shimokawa I., Masoro E.J., Ikeda T.: Duration of dietary restriction: An important determinant for the incidence and age of onset of leukemia in male F344 rats. *J. Gerontol.* 49: B239-B244, 1994.
18. Higami Y., Yu B.P., Shimokawa I., Bertrand H., Hubbard G.B., Masoro E.J.: Anti-tumor action of dietary restriction is lesion-dependent in male Fischer 344 rats. *J. Gerontol.* 50A: B72-B77, 1995.
19. Elwell M.R., Stedham M.A., Kovatch R.M.: Skin and subcutis. In: Boorman G.A., Eustis S.L., Elwell M.R., Montgomery Jr. C.A., Mackenzie W.F. (Eds.), *Pathology of the Fischer rat*. Academic Press, Inc., San Diego, 1990, pp. 261-277.
20. Lagakos S.W.: An evaluation of some two-sample tests used to analyze animal carcinogenicity experiments. *Util. Math.* 21B: 239-260, 1982.
21. Kaplan E.L., Meier P.: Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* 53: 457-481, 1958.
22. Gross A.J., Clark V.A.: *Survival distributions: Reliability applications in the biomedical sciences*. Wiley, New York, 1975.
23. Ross M.H., Bras G.: Influence of protein under- and overnutrition on spontaneous tumor prevalence in the rat. *J. Nutr.* 103: 944-963, 1973.
24. Roe F.J.C., Lee P.N., Conybeare G., Kelly D., Matter B., Prentice D., Tobin G.: The biosure study: Influence of composition of diet and food consumption on longevity, degenerative diseases and neoplasia in Wistar rats studied for up to 20 months post-weaning. *Food Chem. Toxic* 33: 1S-100S, 1995.
25. Carroll K.K., Khor H.T.: Dietary fat in relation to tumorigenesis. *Progr. Biochem. Pharmacol.* 10: 308-353, 1975.
26. Rogers A.E., Longnecker M.P.: Dietary and nutritional influences on cancer: A review of epidemiologic and experimental data. *Lab. Invest.* 59: 729-759, 1988.
27. Moon T.E., Micozzi M.S.: *Nutrition and Cancer Prevention*. Marcel Dekker, Inc., New York, 1989.
28. Henderson B.E., Ross R.K., Pike M.C.: Toward the primary prevention of cancer. *Science* 254: 1131-1138, 1991.
29. Lutz W.K., Schlatter J.: Chemical carcinogens and overnutrition in diet-related cancer. *Carcinogenesis* 13: 2211-2216, 1992.
30. Weinstein I.B.: The origins of human cancer. Molecular mechanism of carcinogenesis and their implications for cancer prevention and treatment. *Cancer Res.* 48: 4135-4143, 1988.
31. Fearon E.R., Vogelstein B.: A genetic model for colorectal tumorigenesis. *Cell* 61: 759-767, 1990.
32. Harman D.: Aging: A theory based on free radical and radiation chemistry. *J. Gerontol.* 11: 289-300, 1956.
33. Cerami A.: Hypothesis: Glucose as a mediator of aging. *J. Am. Geriatr. Soc.* 33: 626-634, 1985.
34. Monnier V.M.: Nonenzymatic glycosylation, the Maillard reaction and the aging process. *J. Gerontol.* 45: B105-B111, 1990.
35. Kristal B.S., Yu B.P.: An emerging hypothesis: Synergistic induction of aging by free radicals and Maillard reactions. *J. Gerontol.* 47: B107-B114, 1992.
36. Laganiere S., Yu B.P.: Effects of chronic food restriction in aging rats. II. Liver cytosolic antioxidants and related enzymes. *Mech. Ageing Dev.* 48: 221-230, 1989.
37. Lee D.W., Yu B.P.: Modulation of the free radicals and superoxide dismutase by age and dietary restriction. *Aging Clin. Exp. Res.* 2: 357-362, 1990.
38. Koizumi A., Weindruch R., Walford R.L.: Influences of dietary restriction and age on liver enzyme activities and lipid peroxidation in mice. *J. Nutr.* 117: 361-367, 1987.
39. Masoro E.J., Katz M.S., McMahan C.A.: Evidence for the glycation hypothesis of aging from the food-restricted rodent model. *J. Gerontol.* 44: B20-B22, 1989.
40. Haley-Zitlin V., Richardson A.: Effect of dietary restriction on DNA repair and DNA damage. *Mutat. Res.* 295: 237-245, 1993.
41. Chung M.H., Kasai H., Nishimura S., Yu B.P.: Protection of DNA damage by dietary restriction. *Free Rad. Biol. Med.* 12: 523-525, 1992.
42. Tong C., Fazio M., Williams G.M.: Cell cycle-specific mutagenesis at the hypoxanthine phosphoribosyltransferase locus in adult rat liver epithelial cells. *Proc. Natl. Acad. Sci. USA* 77: 7377-7389, 1980.
43. Sinha D.K., Gebhard R.L., Pazik J.E.: Inhibition of mammary carcinogenesis in rats by DR. *Cancer Lett.* 40: 133-141, 1988.
44. Lagopoulos L., Sunahara G.I., Wurzner H., Dombrowsky I., Stalder R.: The effects of alternating DR and AL feeding of mice on the development of diethynitrosamine-induced liver tumors and its correlation of insulinaemia. *Carcinogenesis*