Mechanisms of Caloric Restriction Affecting Aging and Disease[a]

ANGELO TURTURRO,[b] KENNETH BLANK,[c]
DONNA MURASKO,[d] AND RONALD HART[b]

[b]*Division of Biometry and Risk Assessment*
National Center for Toxicological Research
Jefferson, Arizona 72079

[c]*Department of Pathology*
Hahnemann University
Philadelphia, Pennsylvania 19102-1192

[d]*Department of Microbiology*
Medical College of Pennsylvania
Philadelphia, Pennsylvania 19129

INTRODUCTION

Caloric restriction (CR) is an experimental paradigm which is thought to slow the onset of aging in a number of different strains and species.[1] A recent Gompertzian analysis confirms this.[2] The mechanism of this alteration is unknown, but hormonal alterations,[3] changes in total metabolism,[4] inhibition of free radical induction,[5] increase in brain size relative to the body,[6] and improvement in immune function[7] have all been suggested to play a role.

Although there have been a number of attempts to define physiological aging outside of the framework of mortality, few if any parameters have been generally accepted as a surrogate for survival on test, especially survival of the longer-lived portion of the population.[1] Since mortality is almost always a result of some disease process(es), the mechanisms of aging can be considered to be the mechanisms of the diseases that result in animal mortality. CR modulates these disease processes differentially, and so is a useful tool to understand the mechanisms involved in disease etiology, as well as a model for their modulation by interventions.

METHODOLOGICAL CONSIDERATIONS

One methodological consideration is the method used to gauge the extent of the restriction used. The most common method is to compare the calorie consumption (or food consumed) to a concurrent *ad libitum* (AL) control, and express the test group as a percent restriction. This method implicitly assumes that the ad lib controls are similar to one another. The validity of that assumption can be seen

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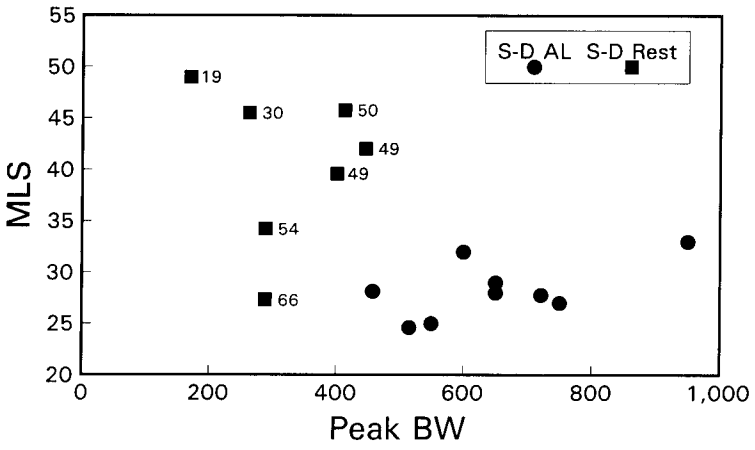

FIGURE 1. Peak body weight (BW), maximal life span and the percent of *ad libitum* calories consumed (*numbers near filled squares*) in experiments using Sprague-Dawley rats. Note the similarity in body weight for a number of 50% restriction studies and the ad libitum controls (*filled circles*) for other studies. Also note the wide variability in the ad libitum control body weights. (From Table 2.4 in Ref. 5, supplemented by information on males from their reference 6.)

in FIGURE 1, which compares different experiments in CR (using different diets and conditions) in Sprague-Dawley (S-D) rats. Using peak body weight (usually the weight near the end of an experiment) as a biological metric, it can be seen that the AL controls in some experiments were similar in weight to the 50% restricted animals used in others. The reason for this is the variability in the AL controls, which can vary between 400 and almost 1000 grams. The larger body weights tend to be associated with poor survival (accelerated aging?). The trend in recent experiments is for S-D body size to increase, which has caused serious problems in drug testing because these tests require good survival at 24 months of age, which is increasingly difficult to achieve.[8] This is occurring also in F-344 rats,[9] as well as other strains.

One method to gauge the intensity of CR is to quantitate its effect on body weight (BW). However, body weight is not a constant during the life span. For example, in FIGURE 2, it is evident that the BW ratio of AL (*e.g.*, NAM) to CR (*e.g.*, NRM) will depend on the age at which the ratio is taken. Peak body weight is probably not a good parameter to use since it occurs late in life, when a good deal of pathology is evident in the population,[10] and pathology can alter body weight. For aging studies, which are quantitated on mortality, a good biomarker for CR is probably one in which it relates to mortality. Previous work (for example, Ross *et al.*[11] in S-D rats) has suggested that early body weight is one correlate of survival. In a series of studies done by the National Toxicology Program (NTP), one can correlate survival at 24 months (when the studies are terminated) to body weight at different ages. Unlike in S-D rats, early body weight (*e.g.*, at 14 weeks of age, BW14W in FIG. 3) is not a good predictor of survival. However, body

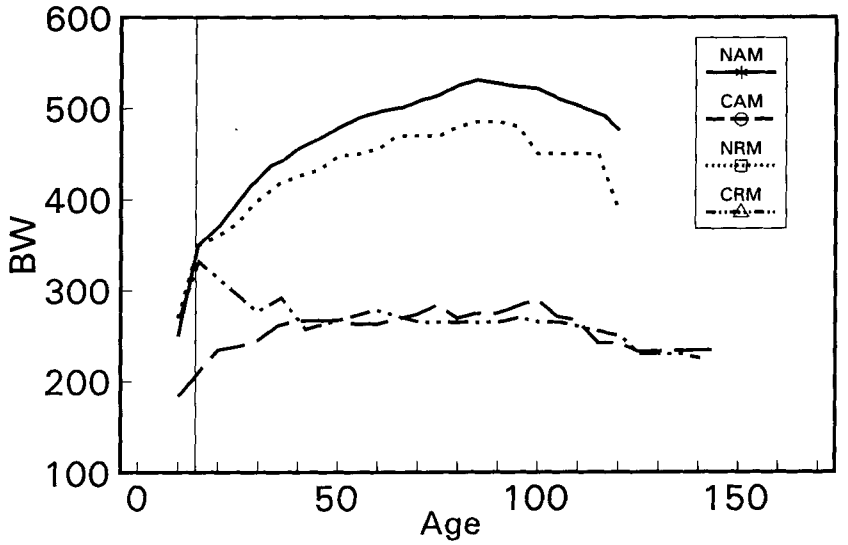

FIGURE 2. BW and diet in F-344 males. NAM is fed NIH-31, NRM is fed 60% of calories starting at 14–16 weeks of age, CAM is fed Masoro Diet C, and CRM is fed 60% of calories starting at 6–8 weeks of age. Husbandry conditions are as reported in Ref. 23.

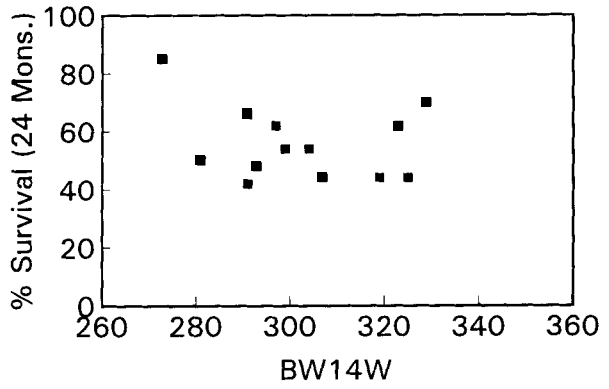

FIGURE 3. BW at 14 weeks of age and survival at 24 months on test (25.5 months of age) for 17 National Toxicology Program (NTP) feed studies with no exposure to agent. Note the lack of a correlation. Data are derived from the latest available feed studies from the NTP Technical Reports (Carcinogenicity Bioassays) Series, *i.e.*, TR419, TR412, TR407, TR387,TR365,TR341, TR337, TR333, TR324, TR322, TR320, TR315, and TR309.

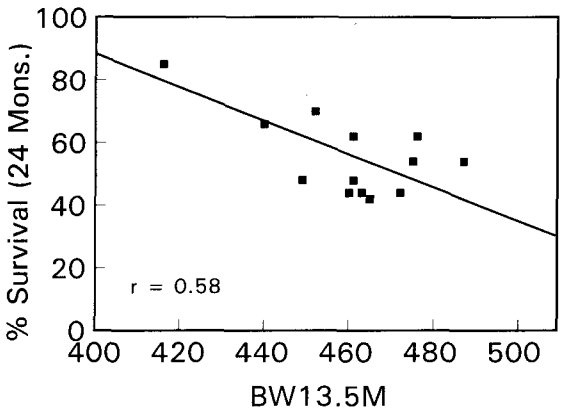

FIGURE 4. BW at 13.5 months of age and survival at 24 months on test (25.5 months of age) for 17 National Toxicology Program (NTP) feed studies with no exposure to agent. Correlation coefficient of 0.58 is highly significant. Data from same studies as in FIGURE 3.

weight at 13.5 months (BW13.5M in FIG. 4) has a significant correlation 0.58 ($p < 0.01$) with survival at 24 months. Using a metric such as BW13.5M for F-344 rats to indicate level of restriction also has the advantage of integrating long-term effects over a significant portion of the life span. It is also interesting that for S-D and F-344 rats, a BW13.5M of approximately 280 grams has been commonly used in restriction experiments (although with very different percent restrictions).

Effects of CR on Disease

The relationship of BW to survival suggests that there may also be some effect of BW on disease. Shown in FIGURE 2 is the BW in F-344 rats kept on different diets. NAM were fed NIH-31, a cereal-based diet similar to that used in NTP studies, while CAM were fed Masoro Diet C, a sugar-based diet. The animals consumed the same amount of calories, though it is not clear that the calories were equally bioavailable. FIGURE 5 shows the effect of CR (60% of calories, BW12M 280 g) on the incidence of leukemia in males. For the NAM, and restricted NRM, there is no effect of CR on the incidence of leukemia. However, for the CAM and CRM, CR appears to significantly decrease the incidence of disease. This is corroborated by the work of Shimokawa *et al.*,[12] who show that CR retards the onset of leukemia in F-344. The effect is not a result of diet since the AL incidences are the same. However, CRM was started at 6–8 weeks of age (shown by the BW differences in FIG. 2), 8 weeks before the NRM studies. This is supported by the evidence from the NTP studies that BW14W is important to the leukemia incidence seen in F-344 rats (FIG. 6).

Thus, the events important to leukemia incidence later in the life span have occurred in the time period before the NRM restriction. This appears to be the age "window" important to the spontaneous leukemia induction and expression.

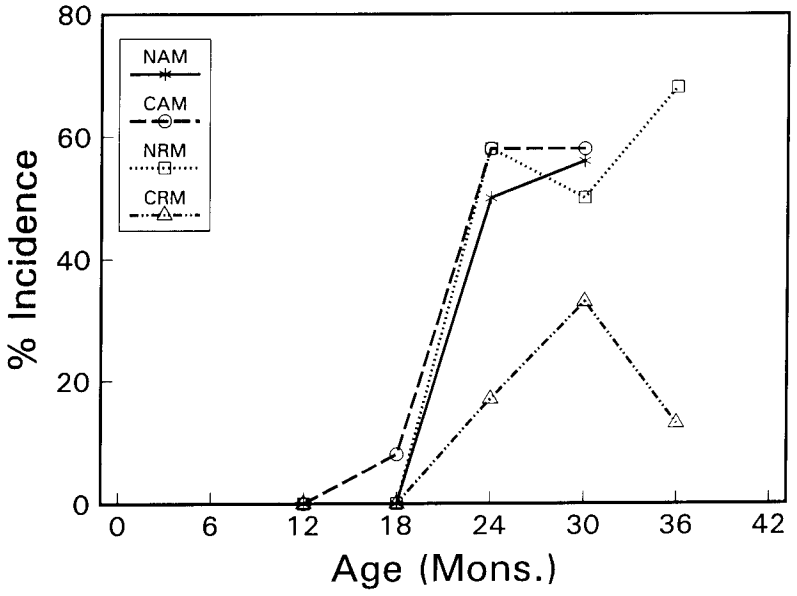

FIGURE 5. Leukemia incidence and diet. Same groups as in FIGURE 2. Incidence is taken from 12 animals at each age, as described in Ref. 24. Note the effect in the CRM group.

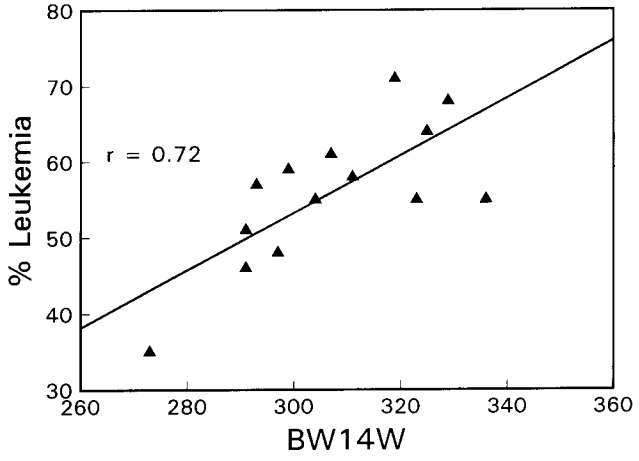

FIGURE 6. Relationship of BW at 14 weeks of age and leukemia incidence at 24 months on test from NTP studies. Correlation coefficient of 0.72 is highly significant. Data are from same studies as in FIGURES 3 and 4.

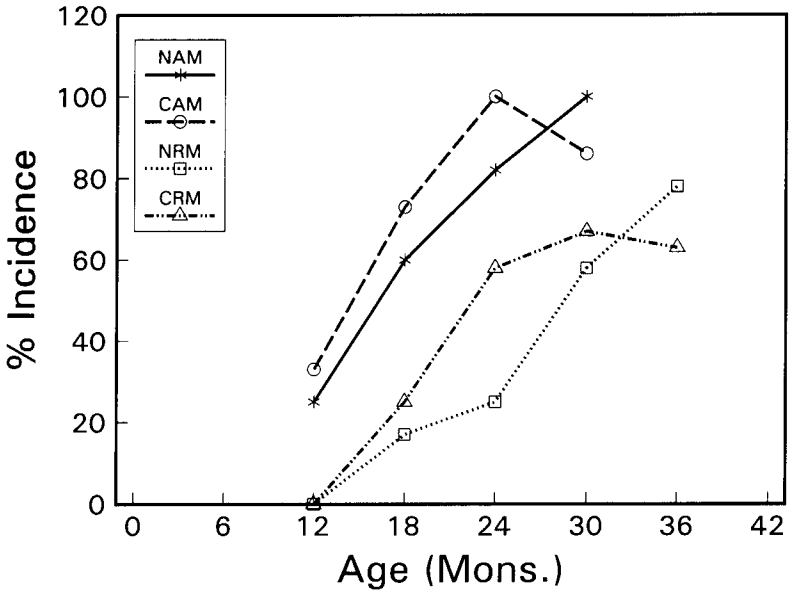

FIGURE 7. Pituitary adenoma incidence and diet in male F-344. Same groups as in FIGURES 2 and 5. Note the effects in both NRM and CRM.

With the other major pathology in F-344 rats, pituitary tumors, the situation is quite different. FIGURE 7 shows the effect of CR on this incidence. It can be seen that there is an effect in both CR and AL groups, with delayed incidence in the animals fed NIH-31. This effect may be diet, or it may be related to the lower weight seen at older ages in the NIH-31-fed groups. Data from the NTP studies (FIG. 8) suggest that the latter may be the case, since they show a positive correlation between BW at later ages (13.5 months) and the presence of pituitary adenoma. This appears again to be different than the S-D rats studied by Ross *et al.*, in which the incidence of pituitary adenoma was affected by restriction early in life.[11]

If one plots the cumulative incidence of animals dying with pituitary tumors and leukemia (FIG. 9), one sees that the pituitary disease appears first, with leukemia occurring later. This suggests that the reason that survival correlates well with BW13.5M in the F-344 rat is that BW13.5M correlates well with the onset of the first fatal disease, pituitary tumors, in this strain. And a similar relationship between pituitary adenoma and survival is found in S-D rats, although BWs at early ages appear to be more important. S-D rats have a much more rapid rise in BW than F-344 rats, leading to high incidences of pituitary tumors at an early age, and poor survival. This may account for the greater sensitivity of the survival curve in S-D rats to an early weight gain than in F-344.

In male B6C3F1 mice, BW14W is a much better correlate of survival than BW13.5M.[13] In this mouse, as well as in the B6D2F1 and C57BI6 mouse, the

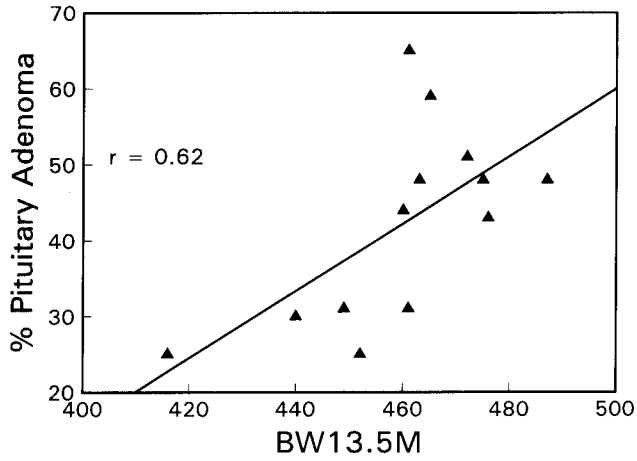

FIGURE 8. Relationship of BW at 13.5 months of age (12 months on test) of age and pituitary adenoma incidence at 24 months on test from NTP studies. Correlation coefficient of 0.62 is significant. Data are from same studies as in FIGURES 3, 4 and 6.

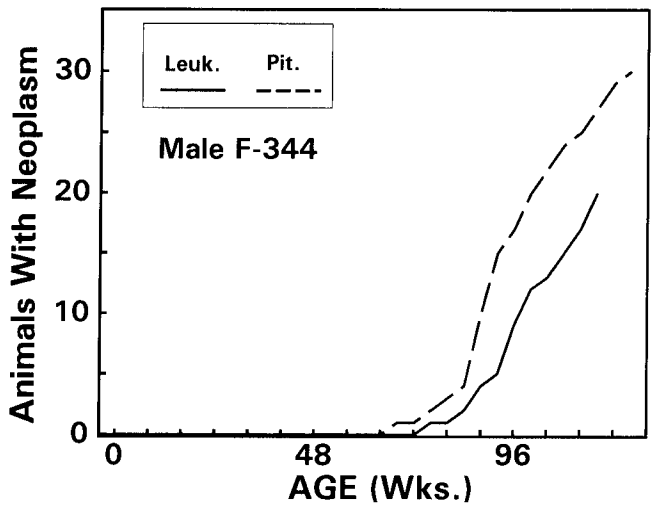

FIGURE 9. Age of spontaneous death and presence of two neoplasms, pituitary adenoma and leukemia, in F-344 male rats on NIH-31 diet, AL and 60% CR (BW13.5M 280). Note that pituitary adenomas arise sooner than leukemias in animals dying spontaneously, consistent with increasing incidence (FIG. 7). Incidence (FIG. 7) is presence of disease in living animals.

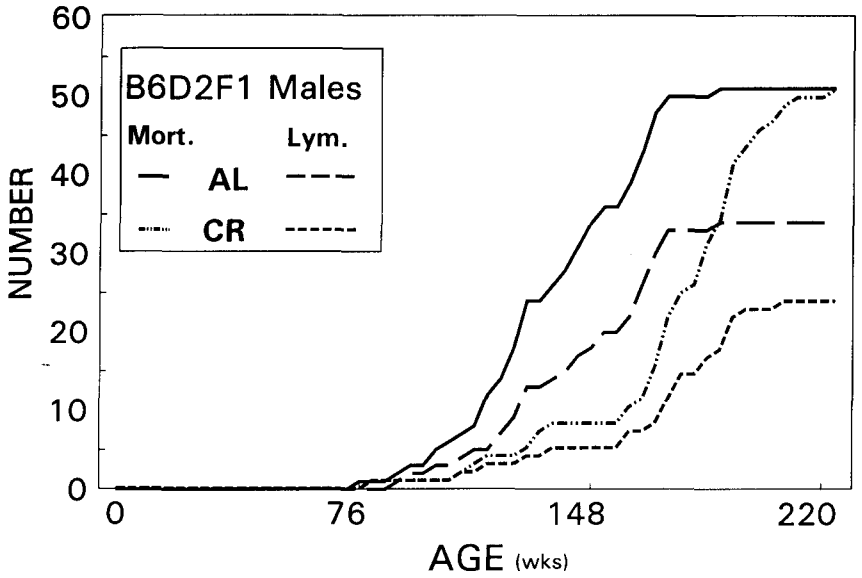

FIGURE 10. Age of spontaneous death and presence of lymphosarcoma in B6D2F1 male mice on NIH-31 diet, AL and 60% CR. Note the general similarity between the slopes of the curves.

major fatal disease that arises is lymphoma, both lymphocytic and histocytic. As for rat granulocytic leukemia, there is a suggestion for a correlation of lymphoma with BW14W. However, as suggested by the close comparison of the mortality curve and the cumulative occurrence of the disease in the B6D2F1 mouse (FIG. 10), the CR effect on lymphosarcoma incidence appears to be the main factor in the effect of CR on survival and may be the important factor in the relationship of early BW and survival.

It appears that any variation in husbandry conditions, treatment, or animal care can alter BW at different times in the life span. These changes can influence survival, and seem to be correlated with neoplastic disease prevalence depending on when in the life span the changes occur.

Possible Mechanisms

The relationship of BW and neoplastic disease is probably not causal. A model to help understand possible mechanisms is shown in FIGURE 11. In this model, derived from toxicity studies,[14] a possible effect of agent is shown in the area labeled initiation. The major factors in promotion and progression, and what appear to be the major factors in neoplastic disease, are the levels of growth factors, hormones, autocrine factors, oncogene expression, etc. The body weight can be thought to be a rough index of these parameters. For example, mammary tissue size is related to prolactin levels,[15] body size to growth hormone,[16] muscle size to testosterone, etc. and body weight adds the effect of all these factors, at

different times in the life span when these factors are most active. For instance, the BW14W may reflect some levels early in sexual maturation, while the BW13.5M may reflect the accumulated effects of a long-term stimulus on an organ, such as the pituitary. This model is consistent with the work on CR which has shown changes in hormones, such as testosterone, glucocorticoids,[17] and prolactin,[15] with CR.

A model system to study this is being developed in the major disease of the mice, lymphoma. In mice, an important factor in the development of lymphoid tumors is the action of an endogenous virus.[18] Preliminary results by two of us (K.B. and D.M.) have shown that CR inhibits the age-related increase in expression of the retrovirus (TABLE 1). The hypothesis being tested is that initial expression of endogenous retrovirus results from recombination of the virus with other endogenous viruses. The control of this expression may be through delay in the production of the recombinant virus, or some effect on transformation and growth. The effect on transformation and growth is probable since there is a significant effect of sex on expression of lymphoma (FIG. 12), with CR inhibiting expression in both sexes. However, this model will allow ready testing of the effects of various factors in the expression of virus.

The effect of CR on neoplastic disease is paralleled by effects on other degenerative diseases. For example, CR delays the incidence of heart disease in rat through what appears to involve stress protein induction[19] and kidney disease, which seems

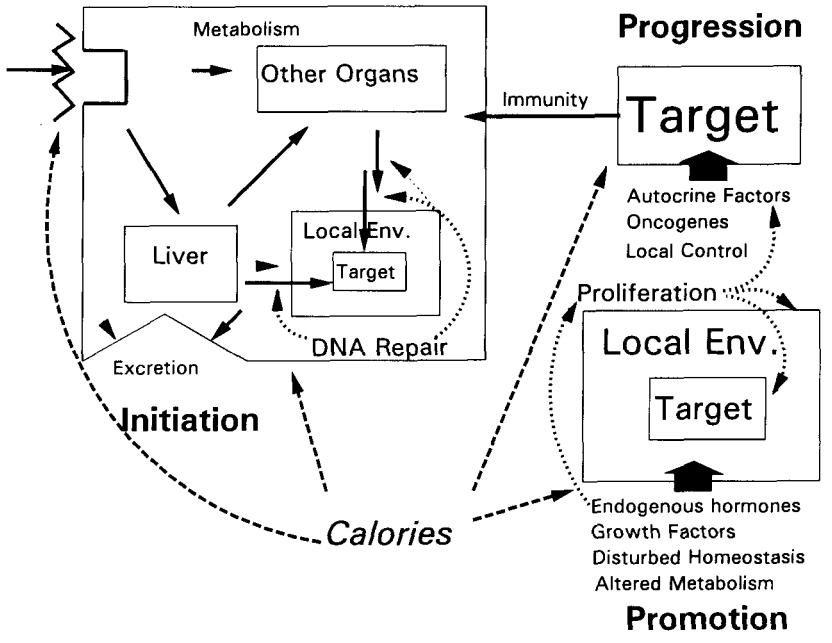

FIGURE 11. A model for the induction of neoplasia with three steps: initiation, promotion and progression. Promotion and progression are the steps most involved in spontaneous disease. DNA repair modifies initiation, proliferation modifies promotion, and immune response modifies progression. All these steps are modified by CR.

to involve protein levels.[20] These mechanisms tend to be accumulative, *e.g.*, when CR was done for only a short time, kidney disease was not much affected.[21] In considering degenerative disease, it is interesting that CR tends to mimic some of the physiological changes that occur with aging. For instance, the level of activity is lowered for most of the day,[22] similar to the lowered activity occurring in aged animals. There is a demasculinization and defeminization of the sex-specific isoenzymes in the liver,[17] similar to what occurs with aging. One way to look at these changes is to see them as adaptations which occur to allow animals to survive to advanced ages, *e.g.*, lower exposure to a toxic protein results in less wear and tear on the nonreplicating nephrons in the kidney.

Taken together, these results suggest that caloric modulation acts to change the induction of specific neoplastic diseases by altering some stage in the development of the disease, affecting its expression later in the animal. Which diseases are affected depends on the particular conditions of the modulation. Chronic diseases are also affected, usually through some change resulting in less damage to nonreplicative structures. Some diseases are hardly affected at all, such as the unknown disease that kills DBA/2N mice early.[13] Lasting through the different life span stages in which the factors important in later disease development occur, inducing changes in physiology similar to those seen with aging, and reducing total exposure to some damaging factors for degenerative diseases, long-term CR will inhibit the onset of almost all major diseases, and this may be why CR slows "aging rate" so consistently.

Implications

The effect that CR has on a number of diseases in rodents should also occur in humans, since the diseases are similar in many ways to their human counterparts. Some diseases, such as the pituitary tumors and the degenerative diseases, seem to be affected at any time in the life span, and appear to be modifiable by restriction at any age, while others seem to be unaffected after certain ages, and may be refractory to modification by CR.

Approaching the effects of CR on a disease-by-disease basis holds promise in dissecting the various mechanisms involved in its action. It also allows for better understanding of the effects in man, since it is mechanism which can be extrapolated, not paradigm.

TABLE 1. Retrovirus Expression in C57Bl/6 Mice[a]

Age (Mons.)	Sample	AL (No. Positive/No. Examined)	CR (No. Positive/No. Examined)
10	spleen	0/5	0/5
	tail	0/4	0/5
16	spleen	1/5	0/5
	tail	0/5	0/5
28	spleen	5/10	1/5
	tail	6/10	1/10

[a] Frozen tail or spleen samples were homogenized in the cold and prepared as a 10% suspension. The clarified suspension was added to SC-1 cells. Four days later, the supernatants were removed and assayed for reverse transcriptase activity.

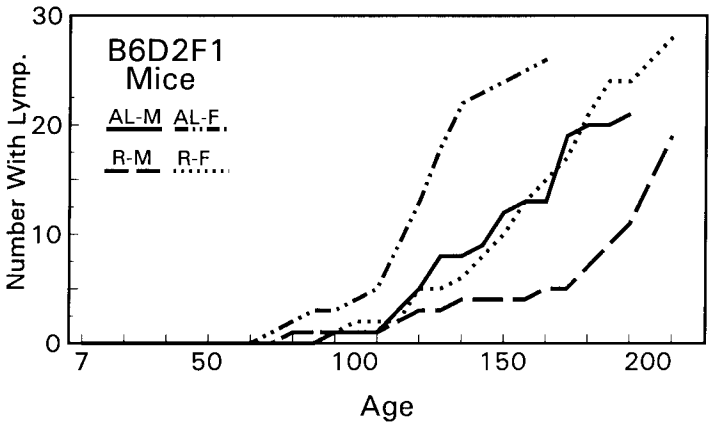

FIGURE 12. Age of spontaneous death and presence of lymphosarcoma in male and female B6D2F1 mice on NIH-31 diet, ad libitum and 60% CR. Note the similarity of the AL male to the CR female, and the strong inhibition of neoplasm in the CR male.

SUMMARY

Caloric restriction (CR) appears to affect aging by the inhibition of the specific chronic diseases which occur at increasing frequency with age. A common disease in F-344 rats, granulocytic leukemia, appears to have a window where it is sensitive to the effects of CR. Other diseases, such as pituitary adenomas, appear to have a different relationship to growth in the animal. Additionally, a model for the major disease for a number of long-lived strains of mice, lymphoma, which CR effects by inhibiting the expression of the causative agent, is being developed. Evaluation of the effects of CR on neoplasia, degenerative disease and physiological parameters suggests that the major factors in expression of these diseases is the alteration of growth factors, hormonal status, etc., and that these alterations also affect strain-specific pathologies depending on when they are changed in the life span. Effecting different diseases at different times in the life span, long-term CR, by limiting exposure to endogenous growth factors, altering physiological characteristics, and limiting exposure to food toxicants, inhibits the onset of disease, and its sequela, aging.

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