Longevity-Assurance Mechanisms and Caloric Restriction

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INTRODUCTION

Aging is a multifocal phenomenon[1] with a genetic base, modified by environment, as are the many diseases falling under the category called cancer. To understand how to modulate these phenomena, it is important to understand their origins in an evolutionary and comparative sense. It is thus useful to discuss aging in the context of what has been termed longevity-assurance mechanisms.[2]

In the context of longevity assurance mechanisms, instead of the pathology of aging, the focus is on those processes which result in species longevity. Organisms thrive despite constant assault from endogenous and exogenous sources. It is axiomatic that evolutionary selection operates most strongly on factors important to reproduction. It is unlikely that selection pressure was directed at increasing longevity, especially in primates, since animals are unlikely to survive to old age in the wild, and, if they do, they probably contribute few offspring since postreproductive senescence can occur at less than half the maximal life span. It is more likely that the increase in life span that is thought to have occurred in evolution of the advanced primates[3] is a by-product of the protective mechanisms which have arisen to allow humans to survive to the age of reproduction, as well as other genes which result in reproductive success.

Comparative analysis highlights those factors that correlate with species longevity. Better termed Longevity Related Processes, or LRP, it was found that four factors could account for 80% of the variation in species life span:[4] brain weight, body weight, specific metabolism and body temperature. In addition, DNA repair has been shown to be correlated with species lifespan.[5]

Caloric restriction (CR) is the only paradigm which consistently extends mammalian lifespan, also reducing the age-related incidence of chronic diseases.[6] Previous attempts to address CR in the context of LRPs have considered only one genotype, and focused on the relationship of CR to lifetime energy dissipation.[7] This dissipation is greater in the restricted animal than an ad libitum fed one on a per gram lean body weight basis.[8] This paper uses lifetime studies in different genotypes to address CR in a more comparative sense.

METHODS

We conducted lifetime monitoring of a 100/genotype/sex/diet cohort for the B6D2F1, DBA/2NNia, B6C3F1, and C57B16 mouse, and the BROWN-NORWAY (BN), BN X F-344, and F-344 rat, all fed NIH-31, and, additionally, the

C57B16 mouse fed EM-911a and the F-344 rat fed Masoro Diet C.[9] The restriction involved feeding 60% of the ad libitum diet consumed by a control population, with vitamin supplementation of the restricted feed to bring it up to the ad libitum levels. The animals were singly housed in Specific Pathogen Free conditions. The animals were monitored daily and weighed, and food consumption was measured on a weekly or monthly basis depending on the genotype and age. Standard pathology profiles were developed every six months, and organ weights were obtained. Core body temperature, physical activity, metabolism,[10,11] P-450 isoenzymes,[12] and DNA repair[13] were measured on these animals.

RESULTS AND DISCUSSION

Body Weight

One of the most important LRP is body weight. Using only one genotype/diet as an example, the average body weight of the C57B1/6 on the NIH-31 diet at selected ages over its life span is shown in TABLE 1. The drop-off after 21 months is not a result of selective die-off of large individuals. Almost all the surviving males lose between 10 and 20% of their body weight as well as consume less food (data not shown). In other genotype/diet conditions (*e.g.*, the same mouse fed EM-911A), the oldest males are self-restricted, sometimes to the level of the experimental group at advanced ages. A similar trend is seen in the females, although interpretation is complicated by mammary tumors, which increase body weight. This increase to a maximum and drop-off at the older ages occurs in all the mice genotypes/diet combinations tested, as well as the F-344 rat. It is most prominent in the males, which generally acquire more weight than the females.

It has been found, from studies on P-450 isoenzyme patterns,[12] that sex-specific patterns decline with age in rats, a decline which is slowed in CR animals. The mechanism for this may involve changes in male pattern growth hormone secretion, as this has been found to be important in the control of P-450 isozyme expression.[12] Analogously, body weight can be viewed as part of the male sex-specific response, which declines with age in ad libitum fed animals. As with the P-450 isoenzyme patterns, CR reduces the expression of this response, but allows it to persist longer.

When compared to animals fed NIH-31, the same animals, under identical environmental conditions fed EM-911A are heavier and have a shorter life span (TABLE 1). EM-911A, contains 11% fat and 20% protein, and was developed primarily as a diet to assist breeding. NIH-31 is a standard rodent diet with 5% fat and 18% protein. Interestingly, the restricted body weights were similar for the two diets. The animals fed NIH-31 outlive the mice EM911A, although the restricted animals have similar life spans on either diet. In a sense, therefore, CR may be considered an extension of the diet effect of using a diet which inhibits the sex-specific response, and which also limits reproduction.

Thus, the same process which increases breeding success leads to decreased longevity. This is an example of what has been termed pleiotropism.[14] It appears that the mechanism may be a hormonal one, probably through the sex-related hormones. Both males and females lose their sex-specific response with age, and this process is delayed by caloric restriction. Many of the biomarkers which are altered with CR may undergo a similar trend, following this pattern. Interestingly, mice that undergo CR stop breeding during restriction, while rats experience reduced fertility.[15] Direct measurement of the relationship of body weight and

longevity is complicated,[16] as pathology, especially since tumors, which increase body weight or inanition, which reduce it, cloud interpretation. However, Ross[17] found that factors such as the time to a particular body weight and consumption during some early ages were important in predicting rat longevity. These observations, as well as Bidder's classical hypothesis about the relationship of growth and aging,[18] may actually be another metric to evaluate the lack of expression of developmentally appropriate sex-specific responses.

TABLE 1. Body Weights and Life Span of C57B1/6 Mice with Different Diets[a]

Age (Mo)	Diet			
	NIH-31		EM-911A	
	A	R	A	R
6	35.5	22.9	37.0	24.0
9	38.2	23.2	45.5	23.5
12	41.2	25.0	49.0	26.5
15	42.2	22.5	47.9	25.5
18	42.2	23.2	47.8	27.0
21	40.8	24.9	49.0	27.5
24	40.2	24.9	46.6	26.8
27	39.6	23.6	39.8	26.9
30	34.8	21.5	29.5	23.0
33	35.5	21.5	24.0	24.0
36	34.0	21.5	x	23.5
39	x	21.4	x	22.8
	Life Span (Mo)			
MnLS	28.5	32.2	25.4	33.6
90LS	33.8	41.1	28.9	40.1

[a] Body weights in grams (standard deviation is less than 10%, and is smaller for restricted animals). MnLS is mean life span, 90LS is time to 90% mortality, in months. A is ad libitum, R is restricted.

Brain Weight

Brain weight is fairly proportional to body weight between species.[7] Within a species, in different genotypes and at different weights, the relationship is more complex. From the measurements done in all the mouse genotype/diet combinations listed in the methods section, the brain weight in the four genotypes at different ages is related to body weight with the equation:

$$\text{Br Weight} = a_1 + a_2 * \text{BW}$$

where values for the constants, and representative values at average body weights, is given in TABLE 2.

The brain weight appears to have a component which is independent of body weight (*i.e.*, is the same for all members) as well as a body weight sensitive component. Using average weights at various ages, as shown in TABLE 3, the body weight sensitive component is approximately 20% of the total brain weight in ad libitum fed animals, and 40–50% of the weight in CR animals. Interestingly, if a CR female mouse weighed as much as the average ad libitum male mouse, its

TABLE 2. Effect of Chronic Caloric Restriction on Some Hepatic Drug Enzymes[a]

Enzyme	Sex	Ad Lib. (9 Mo)	Rest. (9 Mo)	Ad Lib. (22 Mo)	Rest. (22 Mo)
P-450 IIC11	M	2.77	1.70	0.07	0.97
Androgen 5a reductase	F	2.10	4.20	19.9	10.4
Corticosterone Sulfotransferase	F	5.00	18.0	20.8	20.1

[a] Rats are F-344 restricted to 60% of ad libitum diet. All values are means of at least 5 rats. SEMs are less than 20% of mean except for 0.07 value, which is 80%. Enzyme activities are nmol/min/mg microsomal protein except for sulfotransferase which are nmol/min/mg cytosolic protein. (Adapted from Leakey *et al.*[12])

brain weight would be 30% larger than is found in the male mouse. *Peromyscus leucopus* is a criticid rodent which is about as large as the average ad libitum fed mouse, but has a brain approximately twice as large, as well as a life span that is about twice as long.[19] Our attempts to calorically restrict this animal beyond a 20% restriction have failed, and many of its physiological parameters, such as body temperature regulation are more similar to a CR mouse than an ad libitum fed one.[20] In our breeding colonies, *P. leucopus* does not breed as well as mice. This criticid may be an example of a rodent which utilizes many of the same LRP that are available to small mice-like rodents.

Specific Metabolism

One of the oldest hypotheses, such as Rubner's, in aging relates aging inversely to specific metabolism.[21] On a per mouse or rat basis, CR mice have a lower metabolism, as shown in TABLE 4. However, in agreement with previous work,[8] on a per gram body weight or lean body weight basis, as also shown in TABLE 4, CR rodents have a similar or slightly higher specific metabolism. In retrospect, a slightly higher metabolism is not unexpected since a thinner body fat insulating layer and smaller size (indicating a higher surface area/body weight

TABLE 3. Brain Weight in Mice[a]

	Brain Weight = $A_1 + A_2 \cdot$ Body Weight			
Group	A_1	A_2	Avg. BW	Avg. BrW
Male: ad lib.	0.4025	0.0026	35.90	0.494
Male: rest.	0.2760	0.0086	22.45	0.468
Female: ad lib.	0.3906	0.0041	27.63	0.503
Female: rest.	0.2261	0.0118	20.80	0.472

[a] BW is body weight and BrW brain weight in grams. Fifteen mice are used for each genotype on test, at 6, 12, 18, 24 and 30 months of age (when the genotype lives that long).

ratio) will cause the animals to work harder to keep their body temperature elevated above ambient. There is also an increased physical activity in the CR animals,[11,12] which also leads to increased energy output. The only mitigating factor is the decrease in average body temperature which occurs in CR animals.[11,12]

Body Temperature

Another major physiological parameter correlated with life span was body temperature. Lowering body temperature is the only other mechanism ever found to increase longevity (and only in poikilotherms[21]). Hibernating animals (whose body temperature was lower than normal at times of the year) were compared with nonhibernating sister species, and found to live longer.[7] Given this evidence, it is paradoxical that Sacher's analysis suggests that an increased body temperature is weakly related to increased life span. Correlation of body temperature with other life-style factors may be important. For instance, in an interspecies comparison, species dwelling in cooler climates (which live longer) may require higher internal temperatures to survive the cold. CR decreases body average body temperatures, approximately 0.8°C in F-344 rats and 1.3°C in B6C3F1 mice,[11,12] and may have similar effects in man.[22]

TABLE 4. Specific Metabolism[a]

	Ad Lib.			Rest.		
Animal	/Animal	/g BW	/g LBM	/Animal	/g BW	/g LBM
B6C3F1 (33 Mo)	90.2	2.82	3.34	75.3	3.23	3.44
F-344 (18 Mo)	463.3	1.02	1.35	308.8	1.22	1.37

[a] Metabolism is in the average consumption/day in ml O_2/h, ml O_2/h-g. Number under animal designation is age in months when evaluated. Animals are males, approximately 10/group. (Adapted from Duffy *et al.*[10] and Duffy *et al.*[11])

Longevity Equation

The four variables above were related to longevity using Sacher's analysis:

$$L = 8 \cdot E^{0.6}/S^{0.4} \cdot 10^{0.025T}/M^{0.5}$$

where L is longevity in years, E and S are brain and body weight in grams, T is body temperature in °C and M is specific metabolism in watts/g. The brain and body weight are often combined together as encephalization (I), where:

$$I = E/S^{2/3}.$$

By dividing the life span predicted for restricted animals by that predicted for ad libitum fed animals, one derives the equation:

$$L_r/L_a = (I_r/I_a)^{0.6} \cdot 10^{0.025(T_r - T_a)} \cdot (M_a/M_r)^{0.5}$$

where the subscript r denotes restricted and a denotes ad libitum. Values for this encephalization ratio for the species on test, at average weights, are given in TABLE 5.

If we evaluate the encephalization and the metabolism, as shown in TABLE 5 for male F-344 rats, one predicts a 6% increase in longevity for CR. Given the inverse relationship of life span to temperature in intraspecies comparisons such as in poikilotherms, if we consider the Sacher equation without the temperature term, the increase in longevity is predicted to be 12%. If the specific metabolism is put on a per gram lean body weight basis, on the premise that the lean body mass is more appropriate when comparing animals in the wild across species (since animals in the wild are not likely to be maintained similar to laboratory rodents), the predicted life span extension is 16% using the interspecies equation. If temperature is excluded, the predicted extension is 22%, which is what is observed (TABLE 5). In mice, also excluding temperature, the predicted life extensions are approximately a third of those observed.

TABLE 5. Life Extension Predicted by Longevity Equation[a]

Animal	I_d	T_d	M_{dbw}	M_{dlbm}	Lp_t (%)	L_p (%)	L_o (%)
F-344	1.406	−0.82	0.84		7	12	21
(18 Mo)	1.406	−0.82		0.99	16	21	21
B6C3F1	1.25	−1.24	0.87		0	7	37
(33 Mo)	1.25	−1.24		0.97	5	13	37

[a] Longevity equation in text. I_d is ratio of the encephalization of the rest./ad lib., T_d the difference between the rest. and ad lib. body temperatures, M_{dbw}, the ratio of the specific metabolism (per body weight) of the ad lib./rest., M_{dlbm}, the ratio of the specific metabolism (per lbm) of the ad lib./rest., L_{pt}, the % longevity extension using the full equation, L_p, the predicted extension without the temperature term, and L_o, the observed longevity extension, taken as the % of the mean life spans rest./ad lib. Animals are males.

In the context of the equation, in rats, the increase in encephalization more than compensates for any increase in specific metabolism that occurs with CR. The increase in encephalization may suggest that a large brain/body weight is important, or may be an indicator that there has been some selection for a factor associated with a larger encephalization, such as delaying puberty by longer retention of mammalian early growth characteristics (I is elevated in early growth). If the latter is true, it has interesting implications for the evolution of the extended human life span.

In mice, the equations predict a much shorter life span extension. This may be a result of the much lower body temperature seen in the mice. The life span of hibernating animals, which spend part of their life span at lowered body temperatures, is also underpredicted by the physiological equation. For these rodents, CR may have less of an effect on the variable associated with the encephalization, and may have its longevity-extending effects operating though its simulation of hibernation, or lower body temperature. Thus, CR may have a differential effect on different species, depending on what LRPs are most stimulated.

DNA Repair

CR stimulates certain metabolic pathways, physical activity (especially around feeding time) and an apparent direct increase in glucocorticoids,[13] as well

TABLE 6. Age and UV-Induced Repair in Cells Isolated from Male F-344 Rats[a]

Age (Mo)	Hepatocytes[b]		Kidney Cells[c]	
	Ad Lib.	Rest.	Ad Lib.	Rest.
5	5.78	—	—	—
13	4.90	5.72	—	—
22	3.05	4.03	2.01	2.36
28	2.65	3.31	1.34	1.93
34	—	3.02	—	1.33

[a] Rest. is 60% of ad lib. diet. All values are dpm per ug DNA for irradiated/unirradiated cells after one hour. Ratios of rest. and ad lib. are significantly different ($p < 0.01$) at all ages and in both types of cells. (Adapted from Weraarchakull *et al.*[24])

[b] Irradiated with 877J/m^2.

[c] Irradiated with 100J/m^2.

as an increase in the corticosterone sulfotransferase,[13] suggesting a chronic elevation of stress hormone. Given these effects, there may be an increase in DNA damage as a result of CR. This is consistent with the results of Randerrath *et al.*,[23] who found an increase in their I-spots with CR. It is not clear what the significance is of these spots. However, CR has an effect in limiting proliferation, a mechanism for diluting DNA damage in cells. There may be an increase in the observable DNA damage seen in cells. If one effect of CR is to stimulate DNA damage, one would predict a compensatory increase in DNA repair.

In cells from kidney and liver of Fischer 344 rats, there was a decline in both control and UV stimulated DNA repair with age (TABLE 6).[24] For both tissues, consistent with Licastro *et al.*,[25] caloric restriction resulted in less of a decline with age.

Skin fibroblasts from Brown Norway, BN, and Brown Norway × Fischer 344 F1 hybrid, BNF1, showed an increase in both rat genotypes of both types of excision repair (TABLE 7).[13] Increased also were the levels of MGAP activity in BN rats. Skin fibroblasts from the B6C3F1 mice were evaluated at different times of day, and their response had circadian changes[13] with the maximum increase less than half the effect seen in the rats (TABLE 7).

Given the differential species response, DNA repair seems to be more related to the parameters in the longevity equation than longevity extension *per se*. An increase in DNA damage would stimulate DNA repair. Another, mutually nonexclusive, possibility is, given the circadian response, that a hormonal or metabolic

TABLE 7. DNA Repair and Caloric Restriction in Rat and Mouse Skin Cells[a]

Inducer of DNA Damage	Brown-Norway		BN X F-344		B6C3F1	
	A	R	A	R	A	R
MMS (0.5 mM)	1.156	1.180	1.404	1.608	n.d.	n.d.
UV (20 J/m^2)	1.375	1.412	2.072	2.750	n.d.	n.d.
Spontaneous[b] (MGAP levels)	0.38	0.65	n.d.	n.d.	0.34	0.46

[a] Rest. is 60% of ad lib. diet; all rats are 18 months of age. Ad lib. vs rest. repair values are significantly different ($p < 0.01$) in all cases. Values are ratios of stimulated versus nonstimulated cells except MGAP levels which are in femtomoles/ug DNA of O^6-methylguanine-acceptor protein activity. n.d. is not determined. (Adapted from Lipman *et al.*[13])

[b] Mouse levels are at 12 hours post lights on.

adaption triggers this effect. One of the most consistent responses to CR is the decrease in the levels of a number of circulating hormones, especially the gonadotrophins.[15] Available information suggests that hormones can significantly effect DNA repair. An example of this is the effect of the estrous cycle on DNA repair in mammary gland and uterine DNA repair of a nonmetabolically activated agent, excluding hormonal effects on metabolism. It can be seen that the time in the cycle when estrogen is lowest (diestrus) is the time when DNA repair is highest (TABLE 8).[26,27] The mechanism of this is unknown. Thus, an hormonal effect may be important for the elevated DNA repair seen.

CR results in a number of adaptations which contribute to the total effect. Some of these effects may be positive, others, such as perhaps an increase in DNA damage, negative. The total differential response of different strains and species to CR may be a result of the differing weight of the various LRP activated.

TABLE 8. Estrous and DNA Repair in Sprague-Dawley Rats[a]

Organ	Repair Length (hrs)	Stage Diestrous	Proestrous	Estrous
Mammary	1	0.150[b]	0.150	0.160
Epithelium	8	0.123	0.150	0.162
Uterus	1	0.122[b]	0.072	0.073
	8	0.079	0.086	0.062
Liver	1	0.111	0.077	0.088
	8	0.122	0.097	0.102

[a] 50–53-day-old virgin female Sprague-Dawley rats were given a 50 mg/kg b.w. dose of N-methyl-nitrosourea. Values are ratios of 0^6-methylguanine to N^7-methylguanine. (Adapted from Braun *et al.*[26] and Ratko *et al.*[27])

[b] Repair differences between stages are significant at $p < 0.05$.

CONCLUSIONS

CR seems to trigger many of the LRPs that appear to be significant for species longevity in the animals tested. It is also interesting that the state which CR induces, a hypogonadathrophic, hypothermic, low body weight one, is the same that occurs both at early ages and in long-lived members of the species eating ad libitum. These effects have often been thought either as prematuration or as dyshomeostasis of age. However, maintaining these processes throughout life seems to result in extension. Instead, they may be adaptations which are important to longevity.

Building on previous ideas,[28] one theory of how CR acts can be termed the Adaptive-Longevity Related Process Theory of CR:

1) CR mimics a situation very common in the wild, *i.e.*, food scarity at different times of year, or years on end;

2) A successful species preserves itself during these difficult times by either forming some vegetative stage (*e.g.*, a spore) or adapting the organism through some method so it will live long enough to reproduce when times become better again. Reproduction is often discontinued;

3) The mechanisms used to increase life span are the specific LRPs that the species normally is subject to, and emerge in long-lived members of the species.

The most easily conceptualized candidates for these mechanisms are those directly related to reproduction or are tied to the processes which directly regulate reproduction. However other, more distal phenomena, such as growth hormone pulsatility and physical activity, seem to be important. Delayed growth or sexual maturation may be an important LRP whose selection may result in delayed reproduction, increased brain size and increased longevity, a factor that may have operated in the relatively rapid extension of human life span in prehistory.

4) The sum total effect of CR using current methodologies is to extend life span differentially in different strains/diet combinations. However, there are a number of negative effects of the paradigm, such as elevated physical activity and stress hormones, which may result in increased DNA damage. This may lead to mutation, which may balance the positive effects of CR for the long-term survival of the species.

By understanding how CR induces its positive effects, we can perhaps trigger the LRPs which increase longevity, while minimizing the negative effects of CR. In this manner, we can suggest methods for how to trigger these mechanisms in man significantly improving human health.

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