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BEYOND THE RODENT MODEL: CALORIE RESTRICTION IN RHESUS MONKEYS

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ABSTRACT

Lifespan extension and reduction of age-related disease by calorie restriction (CR) are among the most consistent findings in gerontological research. The well known effects of CR have been demonstrated many times in rodents and other short-lived species. However, effects of CR on aging in longer-lived species, more closely related to humans, were unknown until recently. Studies of CR and aging using nonhuman primates (rhesus monkeys) were begun several years ago at the National Institute on Aging, the University of Wisconsin-Madison, and the University of Maryland. These studies are beginning to yield useful data regarding the effects of this nutritional intervention in primates. Several studies from these ongoing investigations have shown that rhesus monkeys on CR exhibit physiological responses to CR that parallel findings in rodents. In addition, several potential biomarkers of aging are being evaluated and preliminary findings suggest the possibility that CR in rhesus monkeys could slow the rate of aging and reduce age-related disease, specifically diabetes and cardiovascular disease. It will be several years before conclusive proof that CR slows aging and extends life span in primates is established, however, results from these exciting studies suggest the possibility that the anti-aging effects of CR reported in rodents also occur in longer-lived species such as nonhuman primates, strenghtening the possibility that this nutritional intervention will also prove beneficial in longer-lived species, including humans.

INTRODUCTION

Calorie restriction (CR) in short-lived species has been shown consistently to increase median and maximal life span and reduce or delay the onset of a wide range of age-related diseases, including cancer. Studies in rodents have also demonstrated that many physiological functions which decline with age are maintained at more youthful levels in CR animals (1,2). First demonstrated by McCay and colleagues (3), lifespan extension by this nutritional intervention has been confirmed in several hundred studies since then. In recent years it has become clear that rodents subjected to CR not only live longer, but apparently age more slowly as measured by several candidate biomarkers of aging (1,2). Calorie restriction has become one of the most widely used paradigms in experimental aging research. While most CR studies have been conducted using rats. and mice, lifespan extension has also been reported in fish, rotifers, hamsters, guppies, and spiders (1). These studies suggest that CR may be a universal phenomenon and not a laboratory phenomenon limited to rodents. Whether or not CR can extend lifespan and slow aging in longer-lived species, perhaps including humans, remains a major question in contemporary gerontology.

To address the issue of applicability in longer-lived species, in 1987, the National Institute on Aging (NIA) began the first controlled study of CR in nonhuman primates (4). A similar study, begun in 1991 is underway at the University of Wisconsin-Madison (5). A third study has emerged from ongoing obesity and diabetes research at the University of Maryland (UMd,6).

Rhesus monkeys are being used in all three studies of CR in nonhuman primates. One of the most widely used primates in biomedical research, the rhesus monkey offers several advantages as an animal model of human aging. The rhesus monkey has a maximal life span approaching 40 years. This, of course, is much longer than that of rodents making it more comparable to humans, yet short enough to allow completion of longitudinal studies within a reasonable amout of time. One major advantage is the ability to conduct strictly controlled laboratory studies in a species phylogenetically closer to humans than the widely used rodent models. Also, physiological functions, particularly reproductive and neurobiological functions, in monkeys are very similar to humans. As such, the over 250 rhesus monkeys used in these CR studies represent a tremendously valuable resource to the gerontological community not only for CR research, but also for the study of basic mechanisms of aging.

Characteristics of the studies are presented in Table 1. It is apparent that the three studies utilize monkeys whose ages range across much of the rhesus lifespan. The NIA study includes monkeys of several ages from across the lifespan while the UW and UMd studies both

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Table 1. Calorie restriction studies in rhesus monkeys

	NIA	Univ. of Wisc.	Univ. of Md.
Age Range (initiation of CR)	1-2, 3-5, > 15	8-14	11-12
Sex (n)			
long-term	M (60), F (60)	M (30), F (30)	M (16-27)[a]
mechanistic studies	M (28), F (12)	M (16)	—
Total	160	76	27
Diet	Semi-synthetic	Synthetic	Purina Chow/Ensure
fat %	5%	10%	13% (chow), 31% (Ensure)[b]
supplement	yes (premix)	yes (premix)[c]	yes (chewable vitamin)
CR paradigm	30% of[d] control level	30% of individual baseline intake	weight stabilization

[a] The exact number of control animals varies between 8-19 dependent on the experiment, 8 monkeys have been on long-term CR.
[b] Three control monkeys receive Ensure diet.
[c] Supplement has been given since 1994.
[d] Control allotments based on NRC Guidelines (9). Food consumption studies over the course of the study have shown this to approximate ad libitum consumption.

focus on adult onset CR. All three studies were designed originally as longitudinal studies and maintain cohorts on long-term CR. Recently, the NIA and UW studies have added monkeys on short-term studies to focus on possible mechanisms of CR using measurements and markers that are difficult or impossible to assess in a longitudinal design. The numbers of animals on longitudinal and short-term studies are shown in Table 1.

Diets used in each study differ, and these differences are important to consider when comparing data among the monkey studies. Dietary fat source and content are among the major differences which could influence certain findings. As Table 1 shows, the diets differ in fat content (5% NIA, 10% UW, and 13 or 31% UMd.). The primary sources of fat for the NIA and UW diets are soy oil, and corn oil, respectively. The open formula diet used in the UMd study contains fat from a variety of animal sources. More detailed information on the NIA (4,7) and UW (5) diets can be found in previous publications.

Another key difference is the method of CR. Diet regimens for the NIA (4), UW (5) and UMd (6,8) studies have been described in detail elsewhere and are discussed briefly here. The UMd study was designed to prevent obesity by maintaining monkeys at a stable body weight (10-12 kg). Monkeys in this study were weight-clamped to prevent the development of obesity which resulted in a reduction in calorie intake similar to that seen in CR. Monkeys are weighed once a week, and food intake is subsequently adjusted to maintain weight. This protocol has resulted in an average 35% reduction in calorie intake, compared to ad libitum-fed controls (6). Both the NIA and UW studies were designed to restrict calorie intake by 30% compared to controls. Most monkeys in the NIA study were still growing and increasing their food intake when the study began. Therefore, it was necessary to base food allotments on estimates of ad libitum intake according to National Research Guidelines (9). Regular monitoring of food consumption over the course of the study has shown that these estimates have approximated ad libitum consumption. Thus, control monkeys have been eating approximately ad libitum, and CR monkeys receive a daily food allotment that is 30% less than the amount offered to age- and weight-matched controls (4). The UW study began in adult monkeys of full size that had reached a stable weight. Thus, it was possible to determine individual ad libitum intake directly without the need to "predict" intake in young, growing monkeys. The 30% reduction in calories was based on each animal's individual ad libitum intake measured before CR began (5). Due to fluctuations in intake as described by Kemnitz et al. (10), a decision was made several months into the study to adjust the food allotments in CR monkeys to more closely approximate the desired 30% restriction level.

Despite the methodological differences among the three studies, it is important to note that, to date, the majority of findings emerging from them show good agreement, particularly with respect to glucoregulation. This review will focus on four main areas of CR research in monkeys: 1) changes in body composition and development (maturation); 2) bioenergetics; 3) age-related diseases; and 4) biomarkers of aging.

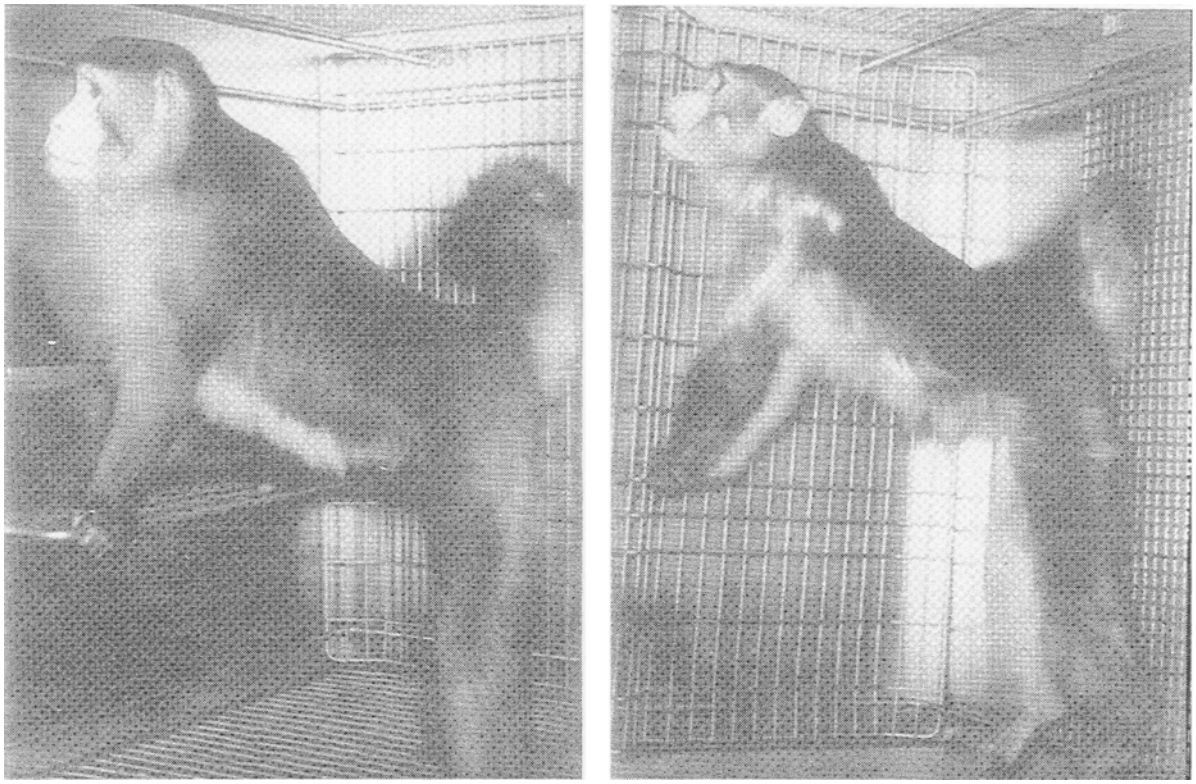
Body Composition and Development

As expected from rodent studies, monkeys on CR experience changes in body composition which, in general, result in smaller body size. Table 2 summarizes published reports of body composition findings from the three studies. A photograph of control and CR monkeys at the NIA (Figure 1) further illustrates effects of CR on body size and composition. Several reports from these studies have documented significant reductions in body weight in CR, compared to control monkeys (5,6,7, 10-12). Furthermore, the rate of body weight gain in young, growing monkeys at the NIA was significantly slower in CR, compared to control monkeys (13,14) suggesting that development was slowed.

Many studies of adiposity (body fat) and lean body mass have been conducted in monkeys subjected to CR using a variety of methods including morphometric measures, isotopic dilution and dual energy x-ray absorptiometry (DEXA). In some early reports, body fat was not altered significantly in CR monkeys (5,11,12). These negative findings were likely related to the duration of CR at the time of measurement, reduced sensitivity of certain adiposity measures, or a lack of statistical

Table 2. Effects of CR on body composition and development.[a]

Study Group	*Body Weight*	*Body Fat*	*Lean Mass*	*Maturation*
NIA	▼[7,11,12]	▼[17]	▼[12,17]	delayed sexual[22] delayed skeletal[7]
U.W.	▼[5,10]	▼[10,16]	▼[16]	—
U.Md.	▼[6]	▼[6,15]	—	—



Control Restricted

Figure 1: Control and CR monkeys from the NIA study. Monkeys are the same age (9 yr.) and had been on study for 9 years when this picture was taken.

power. Most recently, all three studies report reduced adiposity in monkeys subjected to CR for several years (6,10,15-17). Data from the UW study (16) show that during adult-onset restriction, significant reductions in body fat occurred first (at 12 months) followed much later by reductions in lean body mass (36 months). Reduced lean mass in CR monkeys in the NIA study has been confirmed using both isotopic dilution (12) and DEXA (17). In agreement with rodent studies (for review see 1,2), rhesus monkeys on CR are generally smaller, and have significantly lowered body fat and lean body mass amounts, compared to controls.

The slowed rate of body weight gain in rhesus monkeys on CR suggested that CR might have influenced normal development in young monkeys. Studies have shown that, if begun early enough, CR reportedly delays sexual (18,19) and skeletal (20,21) maturation in rodents. The use of fully adult monkeys in the UW and UMd studies precluded assessment of developmental effects of CR. Results from the NIA study agree with findings in CR rodents that both sexual and skeletal maturation were delayed in young monkeys subjected to CR. Roth et al. (22) reported that maturational increases in serum testosterone levels were delayed in CR monkeys by at least one year. Also, Lane et al. (7) reported that CR delayed skeletal maturation as measured by reduced crown-rump length and total body bone mineral content in young adult males subjected to CR. Further evidence of such a delay were data showing that CR delayed (by about 1 year) declines in total serum alkaline phosphatase (7). These findings suggest that, as seen in rodents, both sexual and skeletal development are delayed in young male monkeys subjected to CR. Moreover, there was no evidence that the maturational delays were related to some underlying pathology. Instead, it appeared that maturation proceeded normally, but at a slower rate.

Bioenergetics

Several years of research in rodents, particularly by the University of Texas at San Antonio group, have demonstrated that a reduction in calorie or energy intake is the primary dietary factor responsible for the diverse anti-aging and anti-disease effects of CR (23,24). Total metabolic rate or metabolic rate per unit of metabolic tissue mass could be seen as the link between energy metabolism and life span and this possible linkage supported the development of the "Rate of Living" theory of aging (25). Reduced total energy intake during CR most certainly impacts energy metabolism. Indeed, Sacher (26) and Harman (27) proposed that lifespan extension in this paradigm was due to reduction in overall metabolic rate. The elegant work of McCarter and colleagues (28,29) has shown clearly that a sus-

tained reduction in metabolic rate per unit of metabolically active tissue is not a requisite condition for the prolongevity induced by CR. Some have challenged this viewpoint (30,31), but as McCarter (32) suggested, the longer transient period of reduced metabolic rate seen in these studies most likely relates to the extended period required for reduction in metabolic mass to occur during adult-onset CR.

Studies of energy expenditure in rhesus monkeys on CR support McCarter's explanation and show that metabolic rate (24-hour energy expenditure, 24-h EE) expressed as a function of lean body mass (24-h EE-adjusted) is reduced during adaptation to CR but that the reduction is not maintained after the loss of metabolic (lean) body mass. As seen in the left side of Figure 2, metabolic rate, as measured by 24-h EE-adjusted, decreased as intake was gradually reduced to 30% of ad libitum and remained lowered for 2 months at this intake level. Lean body mass did not change significantly over the same time frame. In a separate experiment, 24-h EE-adjusted in control and CR monkeys was not different after 6 years on CR while lean body mass was reduced significantly in CR, compared to control monkeys. Results from the UW also study support McCarter's view (32) that during adult-onset CR initial reductions in metabolic rate adjusted for body size (mass) persisted for a longer time until the proposed adjustments in metabolic mass occur. For example, Ramsey et al. (16) reported that 24-h EE-adlusted was significantly lower in CR, compared to control monkeys after 24 months and remained lowered until the 42 month measurement. This occurred just after the emergence of significant reductions in lean body mass in CR animals at 36 months. Thus, findings related to energy expenditure from the monkey studies confirm the majority of rodent findings which showed that long-term CR does not result in a sustained reduction in 24-h EE (metabolic rate) and provide additional support for the notion that a reduction in metabolic rate per unit mass is not the mechanism which extends life span and slows aging in this model. The observed reductions do not appear to be related to changes in skeletal muscle (lean) mass as measured by DEXA or isoptopic dilution. However, it is possible that changes in other metaboli-

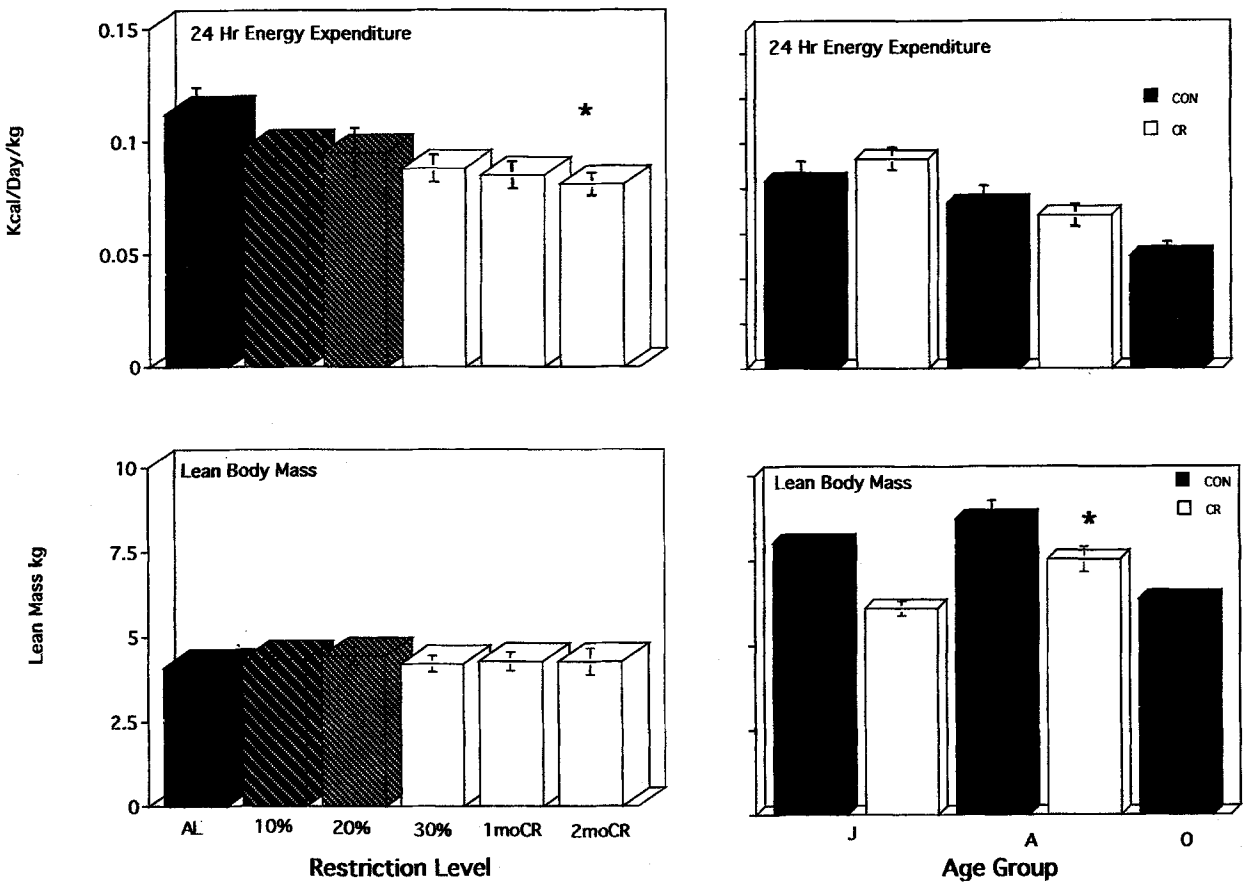


Figure 2. *Effect of CR on Energy Expenditure and Lean Body Mass During Short- and Long-Term CR - NIA Study.* Left-hand panels summarize effects of short-term CR (2 months 30%) and data during long-term CR (6 yr.) shown on the right. Energy expenditure was measured by indirect calorimetry and lean body mass by DEXA. Each bar represents the mean (± SEM) for monkeys at each feeding level (n=5, short-term) or for monkeys in a given age group (long-term). There were no data for CR monkeys in group O. *Indicates significant effect of CR. Reprinted with permission from Lane et al. (41).

cally active tissues such as liver or the gastrointestinal system contribute to the observed reduction in energy expenditure during short-term CR. Measurement of more specific aspects of metabolism such as the metabolic rate of specific organs or tissues, the generation of potentially damaging metabolic products (i.e., free radicals) would be helpful in elucidating the role of metabolism in this experimental model.

Thermoregulation is one such aspect of energy metabolism that might be related to possible metabolic mechanisms of CR and perhaps ultimately to life span. Studies in poilkilothermic species have demonstrated lifespan extension by reduction of ambient temperature (for review see 1). Studies in homeotherms have not yielded similar results. However, reduced body temperature in rodents is a consistent adaptive response to CR (33-38). The reduction of body temperature in CR rodents is similar to the torpor observed in hibernating animals. Studies in both hamsters and reptiles (39,40) have demonstrated a positive relationship between time spent hibernating and life span.

Studies of body temperature in CR monkeys agree with rodent findings and suggest the universality of the thermoregulatory response. For example, Lane et al. (41) reported that, similar to 24-h EE findings, body temperature was reduced (~1.5 °C) during short-term CR (Figure 3). Body temperature was also lowered by CR after 6 years, but as seen in Figure 2, there was no difference in 24-h EE between control and CR monkeys. While it is not known if reduced body temperature relates to the anti-aging effects of CR, these data suggest that metabolic changes metabolism such as reduced body temperature, occur during CR independent of changes in overall metabolic rate per unit of metabolic mass.

Effects of CR on Age-related Disease

Changes in fuel (glucose) utilization have been proposed as one possible metabolic mechanism of CR (23). In addition, changes in glucoregulation induced by CR would also have beneficial effects on age-related diseases, particularly diabetes. Reduced blood glucose and insulin levels have been demonstrated repeatedly in CR rodents (23, 42-44). All three monkey studies have expended considerable effort to characterize glucoregulatory changes during long-term CR in rhesus monkeys (5,6,10,11). The results of this work are summarized in Table 3. Rhesus monkeys subjected to CR in the NIA and UW studies have lower fasting blood glucose and insulin levels compared to controls (10,11) while in the UMd study (6) insulin, but not

Table 3. Glucoregulatory changes in rhesus monkeys subjected to CR.

Study Group	Fasting glucose	Fasting insulin	Insulin sensitivity[a] glucose uptake	Glucose disappearance (K_G)
NIA[b]	▼	▼	——	unchanged
U.W.[c]	▼	▼	▲	unchanged
U.Md.[d]	unchanged	▼	▲	▲

[a] Insulin sensitivity or glucose uptake determined by Minimal Model (U.W.) or euglycemic clamp (U.Md.).
[b] See reference 11.
[c] See reference 10.
[d] See reference 5.

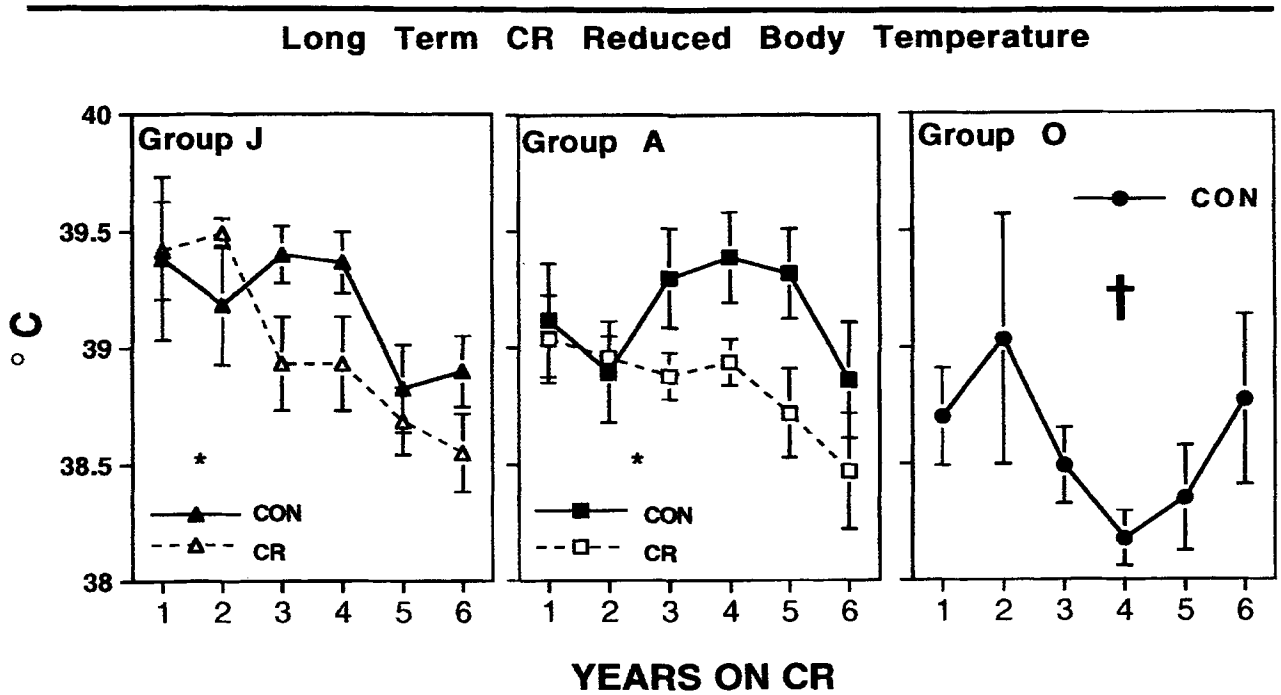


Figure 3. *Body Temperature is Reduced by Long-Term CR.* Each point represents the mean (±SEM) rectal body temperature for monkeys in a given age group. † Indicates significant effect of age group; * indicates significant effect of CR. Reprinted with permission from Lane et al. (41).

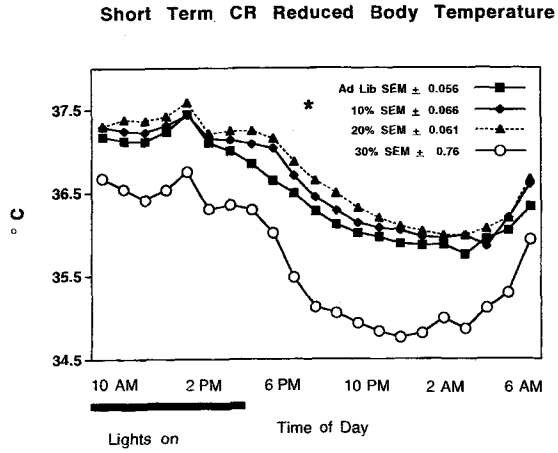


Figure 4. *30% Reduced Body Temperature.* Body temperature was monitored continuously for 7 days using subcutaneous radiotelemeteryimplants. Data represent the mean (n=5) 24-hour body temperature for monkeys at each feeding level. * Indicates significant effect of intake level. Reprinted from Lane et al. (41) with permission.

glucose levels were lower in monkeys subjected to CR. Using the Minimal Model technique, Kemnitz et al. (10) demonstrated that both insulin sensitivity and glucose effectiveness were improved in CR, compared to control monkeys. In the UMd study, euglycemic clamp studies have shown that insulin-stimulated glucose uptake was significantly greater in CR, compared to control monkeys (6). Findings from all three monkey studies support Masoro's suggestion that glucose effectiveness or insulin sensitivity are improved by CR.

Consistency in glucoregulatory effects of CR in the monkeys studies is remarkable when differences in body composition are considered. Specifically, monkeys in the UMd (15) and UW (10) studies have considerably more body fat compared to monkeys in the NIA study (11). Control and CR monkeys in the UMd have approximately 30% and 18%, respectively (15). Control (31.7%) and CR (23.5%) monkeys in the UW study also have considerable amounts of body fat (10). Although somewhat younger, adult monkeys (aged 12-14 years) at the NIA (11) have considerably less body fat 14.3% and 11.7%, for control and CR monkeys, respectively. Despite marked differences in body fat among the studies, the consistency of glucoregulatory responses suggests that effects of CR on glucoregulation are not wholly attributable to changes in body composition such as the reduction of body fat and the prevention of obesity. This supports a more fundamental role of glucoregulation in the CR paradigm. The combined effects of CR on glucoregulation and body composition reported in the monkey studies (6,10,11) are consistent with a decreased insulin resistance, improved glucose tolerance, and a reduction in the occurrence of Type II diabetes and its associated complications. Also, the prevention of hyperinsulinemia would likely result in reduced cardiovascular disease as elevated insulin levels are known to be associated with heart disease (45), hypertension (46) and atherosclerosis (47).

Studies of cardiovascular disease in CR monkeys have not been conducted, however, limited data regarding certain risk factors have been reported and in general support improved cardiovascular disease risk in CR monkeys. In the UW study Kemnitz et al. (5) reported no effect of CR on pulse rate, systolic and diastolic blood pressure, or mean arterial pressure after 12 months. Subsequent reports have not contained blood pressure data and it is not known if, like several other parameters in this study, differences in blood pressure between control and CR monkeys emerged at a later time.

Data regarding other risk factors of cardiovascular disease, such as serum lipid levels and HDL subfractions, suggest an improved risk profile in CR monkeys. For example, reduced triglycerides have been reported in monkeys from all three studies (6,Verdery, RB and Kemnitz, JW, unpublished data). In addition to serum lipids, researchers at the NIA have also assessed the effects of CR on various lipoprotein subfractions. HDL subfraction studies have shown that the HDL subfraction HDL_{2b} was increased in rhesus monkeys subjected to long-term CR (Verdery, RB., unpublished data). Lower serum HDL_{2b} is inversely related to atherosclerosis in humans (48-53) Thus, the significant increase in HDL_{2b} seen in CR monkeys suggests a reduced occurrence of cardiovascular disease. Findings related to serum lipids in CR rodents also suggest improvements in cardiovascular disease risk (54-57). The effects of CR on triglycerides and HDL_{2b}, are unique in that only total calorie intake was reduced, while cholesterol, fatty acid composition, and the proportion of fat, protein, and carbohydrates were not changed.

Data on body composition, glucoregulation, and lipid and lipoprotein levels suggest that CR, well known for extending life span in short-lived species, will reduce or prevent certain age-related diseases, such as diabetes, and will improve risk profiles for others like cardiovascular disease. Thus, even if the marked enhancement of lifespan associated with CR in rodents does not occur in monkeys, it is likely that CR will delay the onset and lessen the impact of several age-associated diseases and leading to moderate lifespan increases.

Biomarkers of Aging

While it is now generally accepted that CR slows the rate of physiological aging in rodents (for review see 1,2), the effect of CR on aging rate in rhesus monkeys has not been established. Given the long life span of rhesus monkeys (approximately 40 yr.) development of reliable biomarkers of aging that could be used to test the effectiveness of CR on reducing the rate of aging in monkeys is of primary importance. To this end a major focus of both the NIA and UW studies (4,5) has been the development and assessment of biomarkers of aging.

Nakamura et al. (58) described a strategy for identifying and testing biomarkers of aging that could be useful for assessing the effects of CR on aging rate in longitu-

dinal studies of aging in nonhuman primates. Detailed development and rationale of the strategy is beyond the scope of this review. Briefly, the major screening criteria included significant cross-sectional and longitudinal correlations with chronological age and significant stability of individual differences. After potential biomarkers were identified, these variables were submitted to principle component analysis to identify which biomarkers loaded significantly onto a component which reflected chronological age. A multivariate index of biological age (biological age score, BAS) was then constructed based on factor loadings of each variable (biomarker) on the principle (aging) component.

The strategy described above was used to test a large number blood chemistry and hematology parameters for their utility as biomarkers of aging (58). Six potential biomarkers were identified: 1) serum glutamic oxaloacetic transaminase; 2) alkaline phosphatase; 3) total protein; 4) globulin; 5) blood urea nitrogen; and 6) phosphates. These variables loaded onto a single component that described over 50% of the variance to indicate marked covariance among the variables. Based on the factor loadings of the 6 candidates, a BAS was calculated for each of 29 monkeys that had been on the CR study at the NIA for over 5 years. There was no statistically significant difference between control and CR monkeys, however, the slope of the regression of BAS onto chronological age appeared to be steeper for control, compared to CR monkeys (Figure 5). Thus, calculation of BAS as described cannot yet demonstrate a clear difference in aging rate in CR monkeys. Assuming a continued linear trend in these data, we can project the emergence of statistically significant differences in this index of the rate of aging in the next several years. In addition to hematology and blood chemistry data, sufficient longitudinal data have now been collected on a variety of other parameters that could serve as biomarkers of aging including bone loss, adrenal steroid levels, immune function, and thermoregulation. Forthcoming studies will evaluate these potential biomarkers and include appropriate markers in the calculation of BAS.

Biomarkers of aging should not only meet the criteria described above, but should also exhibit different rates of change related to species life span, being accelerated in short-lived compared to long-lived species. The accumulation of pentosidine, a product of glycoxidative stress in skin collagen, may be such a marker. In a recent report Sell et al. (59) showed that pentosidine accumulation increased with age in several mammalian species including rhesus and squirrel monkeys from the NIA study. The report also showed that species-specific

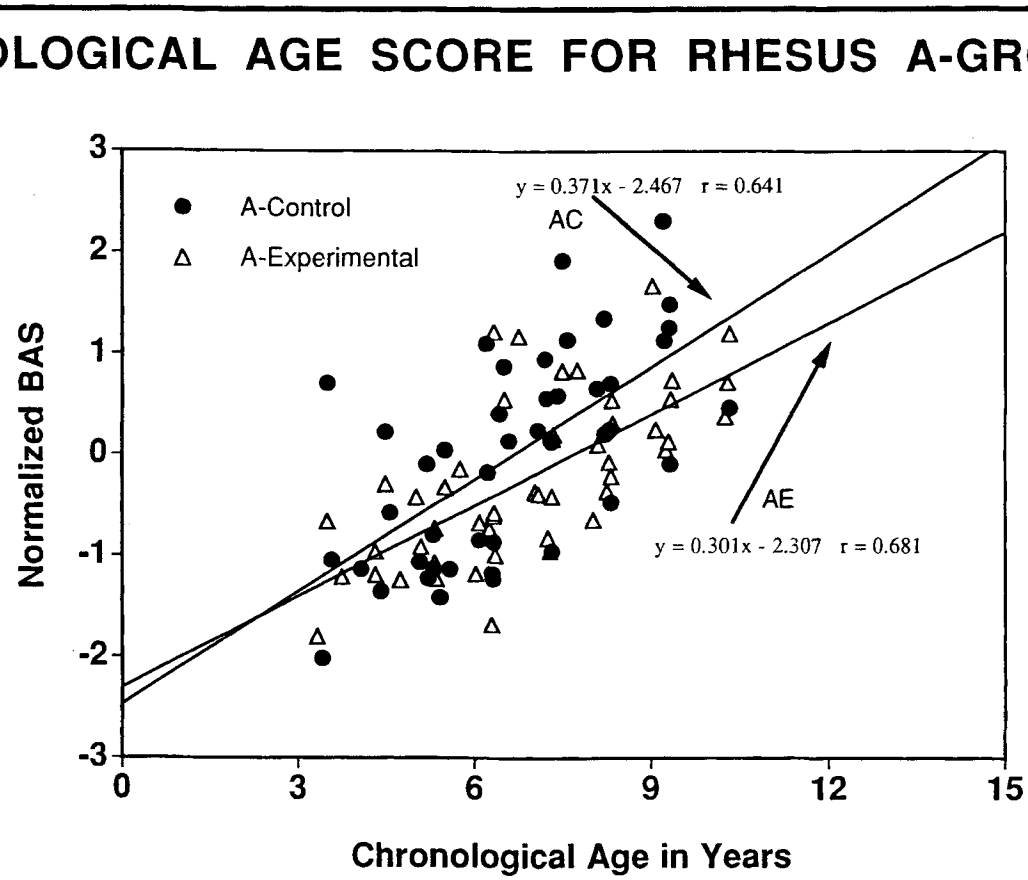


Figure 5. *Effect of CR on Biological Age Score.* The potential effect of CR on biological age is shown by the relationship (linear regression) between biological age score and chronological age. Reprinted with permission from Nakamura et al. (54).

life span and glycoxidation rate, as measured by pentosidine were inversely related and that skin pentosidine levels were lower in Fischer 344 rats subjected to CR. The effects of CR on pentosidine in rat skin collagen provides further evidence that CR slows species-specific aging rate. Studies are currently underway at the NIA to determine the effects of CR on skin pentosidine levels in rhesus monkeys.

Several other biomarkers of skin aging have also been studied in monkeys on CR at the NIA. For example, the rate of wound closure decreased with age, but was not significantly altered in CR monkeys (60). Consistent with these findings, no effect on wound closure has been reported in CR rats (60,61). Studies of fibroblast clonal efficiency using clone size distribution assays have shown that older rhesus monkeys form more small clones compared to younger monkeys. Again, however, there was no consistent effect of CR on clone size distribution (Pendergrass, W, unpublished data). Studies of cellular proliferation in CR rodents have also failed to demonstrate an effect of CR on fibroblast proliferative capacity (62,63) The rate of fingernail growth has been suggested as a possible biomarker of aging in nonhuman primates (64) and has been measured in the NIA monkeys. Preliminary findings suggest that rhesus and squirrel monkeys exhibited the expected age-related declines in nail growth rate (Lane, M, unpublished data) but that CR did not significantly alter the rate of nail growth. To date, none of the markers of skin aging assessed are affected by CR such that aging processes appear to be slowed. However, it is likely that such processes, as the closure of surface wounds, skin cell proliferation or the growth rate of fingernails would remain unchanged during CR, since these processes may not be crucial for survival. Instead, it is more likely that CR would have more marked effects on functions more closely related to life maintenance.

Another well known marker of aging that has been measured in the NIA study is serum levels of dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEAS). These adrenal steroids have generated much interest lately among gerontologists due to their dramatic age-related declines reported in humans (65-66) and primates including baboons (67) and rhesus monkeys (68). Several correlative studies have suggested an association between lower serum levels of DHEA or DHEAS and the incidence of age-related diseases, such as diabetes and cardiovascular disease. Several studies report findings suggesting similarities between elevated levels of these steroids and the effects of CR. For example, DHEA treatment prevented obesity in mice (69), and altered tumorigenesis (70). Furthermore, reduced serum insulin is associated with increased DHEAS and lowered DHEAS is associated with increased cardiovascular disease (66). These effects have also been seen during CR. Indeed, it has been suggested that DHEA and CR might affect age-related disease through similar mechanisms (71). Until recently, no data regarding DHEA or DHEAS levels in CR animals had been reported. Preliminary findings from the NIA study (22, 68) showed that both DHEA and DHEAS levels decline, as expected, with age in male and female rhesus control monkeys. Interestingly, when young male controls began to exhibit declines in serum DHEAS, levels in CR monkeys of the same age remained elevated. These data suggest that CR might slow the age-related decline in DHEAS seen in this species.

SUMMARY

Calorie restriction is the only non-genetic intervention which consistently extends life span and slows the rate of aging in short-lived mammals. Over the past several decades extensive literature on CR in rodents has evolved, however, until recently, little was known about effects of CR in longer-lived species more closely related to humans. In the last 5 years there has been a significant increase in published reports regarding the effects of CR in nonhuman primates. One of the most important conclusions to emerge from these studies is that physiologically, rodents and monkeys on CR respond in a similar manner. A summary comparing findings between rodent and rhesus monkeys on CR is presented in Table 4.

The growing body of evidence from the monkey studies show that CR induces a wide variety of changes in physiological function that are consistent with data reported in rodent studies in which life span has been extended. Furthermore, it is interesting to note that many of these findings, such as reduced glucose and insulin, effects on 24-h EE, and reduced body tempera-

Table 4. Comparison of CR effects in rodents and monkeys[a]

Finding	*Rodents*	*Monkeys*
Body Composition		
body weight	▼[1,2]	▼[5-7,10-12]
body fat	▼[1,2]	▼[5,15-18]
lean mass	▼[1,2]	▼[12,16,17]
Development/maturation		
sexual	▼[18,19]	▼[22]
skeletal	▼[10,21]	▼[7]
Metabolism		
fasting glucose	▼[23,42-44]	▼[10,11]
fasting insulin	▼[13,41-44]	▼[10,11]
insulin sensitivity	▲	▲[10]
energy expenditure (long-term)	no difference[28,29]	no difference[12,16,41]
energy expenditure (short-term)	▼[30]	▼[41]
body temperature	▼[33-38]	▼[48]
locomotor activity	▲[34,35,38]	▼/no change[16,17]
Lipids/lipoproteins		
triglycerides	▼[54-57]	▼
HDL_{2b}	??	▲
Biomarkers/other effects		
wound closure	no change/slowed[62-63]	no change[60]
clonal efficiency	??	no change
DHEAS	??	delayed decline[68]
IL-6	▼[72,73]	▼[7]
IGF-1	▼[75]	▼[174]

[a] Superscript numbers indicate references for specific findings

ture, are consistent with physiological changes that have been proposed as related to possible mechanisms of CR in rodents. This universality across species could be interpreted as evidence that metabolic changes of this sort represent "fundamental" responses to CR and suggest the importance of metabolism in the search for possible mechanisms of the anti-aging and anti-disease effects of CR. It will be several more years before it is known if CR significantly extends life span in longer-lived species, however, several biomarkers of aging have been identified and preliminary findings suggest that CR might affect the rate of aging in rhesus monkeys. The findings to date from studies of CR in rhesus monkeys strengthen the possibility that CR will extend life span and slow aging in longer-lived mammals. It is apparent from the monkey studies that even if the marked extension of life span associated with CR in rodents does not occur in monkeys, this nutritional intervention will reduce or delay the onset of several age-related diseases, such as diabetes and cardiovascular disease, thus improving the quality of life in later years.

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