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Experimental Gerontology 38 (2003) 35–46

Experimental Gerontology

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Calorie restriction in rhesus monkeys

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Received 1 May 2002; accepted 9 May 2002

Abstract

Calorie restriction (CR) extends lifespan and reduces the incidence and age of onset of age-related disease in several animal models. To determine if this nutritional intervention has similar actions in a long-lived primate species, the National Institute on Aging (NIA) initiated a study in 1987 to investigate the effects of a 30% CR in male and female rhesus macaques (*Macaca mulatta*) of a broad age range. We have observed physiological effects of CR that parallel rodent studies and may be predictive of an increased lifespan. Specifically, results from the NIA study have demonstrated that CR decreases body weight and fat mass, improves glucoregulatory function, decreases blood pressure and blood lipids, and decreases body temperature. Juvenile males exhibited delayed skeletal and sexual maturation. Adult bone mass was not affected by CR in females nor were several reproductive hormones or menstrual cycling. CR attenuated the age-associated decline in both dehydroepiandrosterone (DHEA) and melatonin in males. Although 81% of the monkeys in the study are still alive, preliminary evidence suggests that CR will have beneficial effects on morbidity and mortality. We are now preparing a battery of measures to provide a thorough and relevant analysis of the effectiveness of CR at delaying the onset of age-related disease and maintaining function later into life.

Keywords: Aging; Nutritional modulation; Glucoregulation; Biomarkers; Lifespan

1. Introduction

Virtually all information known about the effect of calorie restriction (CR) on aging processes and longevity comes from research conducted with rodents and other short-lived species. In the last decade, results have emerged suggesting that long-lived nonhuman primates (NHP) will benefit similarly from CR. This suggestion comes not directly from mortality results, as the average lifespan for rhesus monkeys, the most widely used NHP model, is around 25 years; thus, it will be several years before current monkey studies can yield significant survival results. Rather, the hypothesis that this intervention will increase lifespan in NHPs receives support from striking parallels between findings from studies in rhesus monkeys on CR with those from rodent studies. These similarities include changes in body composition, maturation and reproduction, metabolism, and the reduction of risk factors for diabetes and cardiovascular disease (reviewed in Lane et al., 1997a). This hypothesis is further supported by findings that CR effectively attenuated the age-related decline in hormones such as dehydroepiandrosterone (DHEA) and melatonin in monkeys.

In 1987 the National Institute on Aging (NIA) initiated a study to determine the effectiveness of the CR paradigm in an animal model closely related to humans. This study began with a group of 30 male rhesus monkeys and was doubled to 60 in 1988. Sixty females were added in 1992. Monkeys ranged in age from 1 to 17 years at the initiation of the study and have been fed either a diet approximating ad libitum (CON) intake or a CR diet that targeted 30% less calories than age- and weight-matched controls for 13–15 years (males) or 10 years (females). This large age range provided a unique opportunity to study CR initiated in juvenile, adult, and old animals. Animal diet and husbandry have been described previously (Ingram et al., 1990; Lane et al., 1992).

While data on morbidity and mortality would provide the most compelling evidence that CR could retard aging and enhance longevity, the maintenance of function—cellular, organ, physiologic, and behavioral—is an equally important component of any aging study. Therefore, the NIA is currently developing a battery of assays to conduct over the next several years that will assess these functions. These data, in combination with morbidity and mortality, will

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0531-5565/03/$ - see front matter
PII: S0531-5565(02)00146-8

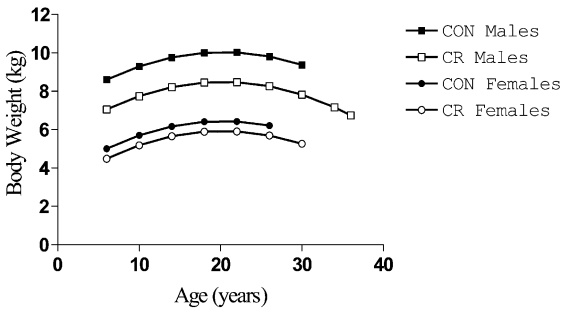

Fig. 1. Mixed effect model of body weight as a function of age in male and female rhesus monkeys on a control diet (CON) or caloric restriction (CR).

address the question as to whether CR is an effective mechanism for maintaining health and prolonging life in primates, including humans.

This paper summarizes findings to date on the effects of CR in NIA rhesus monkeys on several physiological functions that are similar to those observed in shorter-lived species. As well, it addresses the current status of morbidity and mortality of the project and how functional tests of aging will be incorporated to best characterize the effectiveness of this anti-aging intervention.

2. Effect of CR on body size and composition

One of the hallmark effects of CR is reduced body weight and fat, a finding widely reported in rodent literature

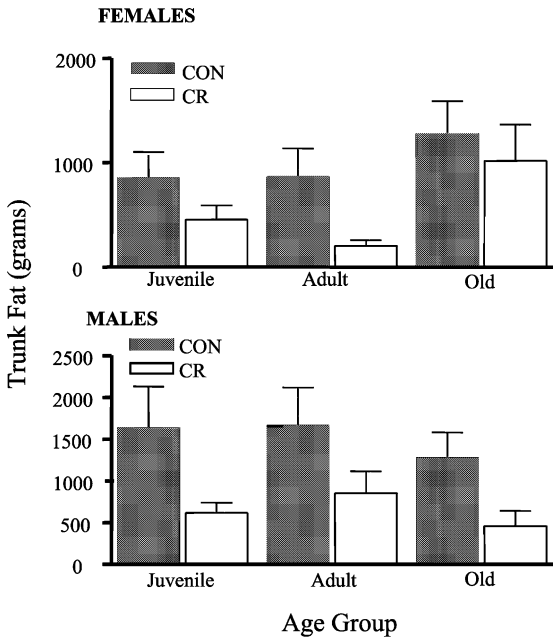

Fig. 2. CR reduces abdominal (trunk) fat. Each bar represents the mean (±SEM) amount of trunk fat determined by dual energy X-ray absorptiometry after 6 (females) or 11 (males) years on CR. Ages at the time data were collected for females: J: 7–9, A: 6–13; O: 22–27 years; for males: J: 12–13; A: 14–16; O: 28–34 years. The effect of CR on reducing trunk fat was significant for both genders ($p < 0.05$). Reprinted from Lane et al. (1999a).

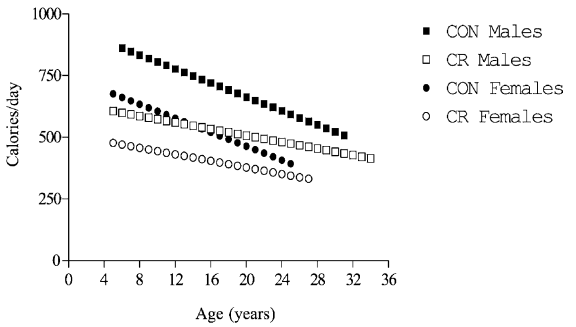

Fig. 3. Mixed effect model of daily caloric intake as a function of age in male and female rhesus monkeys on a control diet (CON) or caloric restriction (CR).

(reviewed by Weindruch and Walford, 1988; Yu, 1994). This result is not surprising considering that generally a 30–40% reduction in caloric intake is imposed. This finding has been confirmed in rhesus monkeys as in general, both male and female CR monkeys weigh less than age-matched controls (Lane et al., 1999a). Interestingly, the effect of CR on reducing body weight was more pronounced in males compared to females (Fig. 1; Mattison et al., unpublished data). The mechanism for this sex difference is unclear but may be related to sex hormones and/or leptin levels. In agreement with rodent studies, rhesus monkeys on CR are also generally smaller with lower body fat and lean body mass than CON (Lane et al., 1992; Weindruch et al., 1995). Further, CR monkeys have less trunk fat and a reduced trunk to leg fat ratio (Fig. 2; Lane et al., 1999a). This reduction in central obesity would presumably reduce the risk for cardiovascular disease and diabetes (Lakka et al., 2001; Pihl and Jurimae, 2001).

Recently, we reported an age-related decline in body weight in both CON and CR groups of male and female monkeys (Fig. 1; Mattison et al., unpublished data). As observed in Fig. 3, a longitudinal decline in caloric intake in adult monkeys might explain this drop in body weight in older monkeys. This age-related decline in caloric intake parallels many reports in humans (reviewed by Morley, 2001). There are several possible explanations for this age-related decrease in dietary intake including but not limited to reduced motivation to eat, decreased energy expenditure or sensory abilities, or changes in blood chemistry. It would appear that rhesus monkeys could serve as a valuable model for studying the mechanism of this change in intake that often results in cachexia in humans due to insufficient nutritional intake.

3. Skeletal health

Early data from the NIA suggested reduced or delayed skeletal development as evidenced by shorter crown-rump measures in CR males compared to CON and an attenuation of the decline in serum alkaline phosphatase (AP), an

osteoblastic product, in fully grown monkeys (Lane et al., 1995a). The change in total AP levels parallels bone growth and development and is lowest at the age when bone growth is complete (Pyle et al., 1971). AP levels in humans were reported to increase during adolescence then rapidly decline to adult levels (Gordon, 1993). This delay in monkeys may be indicative of a slowing of an age-related process. Rodent studies also found that CR delayed skeletal maturation (Kalu et al., 1984a,b).

Recently Black et al. (2001) reported lower bone mineral content and bone mineral density values in rhesus males after 11 years on CR. These differences in male monkeys can be accounted for by lower body mass, particularly lean mass, of the CR monkeys and not a disruption of bone turnover (Black et al., 2001). Similarly, femoral bone mineral content was reduced in CR rats which was also accounted for by a reduction in body weight (Sanderson et al., 1997). This reported diet difference is not without potential risk as smaller body size and lower bone mineral density are associated with increased risk of fragility fractures in humans (De Laet et al., 1998; Ensrud et al., 1997). No evidence of increased fracture has been observed in the NIA study.

In contrast to the changes observed in the male monkeys, bone mass was maintained in CR females compared to CON (Lane et al., 2001). This sex discrepancy in the NIA study may be explained by the older mean and maximum age of the male population or that males were studied after 11 years on CR compared to only 6 years for the females. It was, however, clear that long-term CR did not affect biochemical markers of bone turnover or hormonal regulators of bone metabolism as determined by yearly measures of osteoclacin, 25-hydroxyvitamin D, 1,25-hydroxyvitamin D, and parathyroid hormone (Lane et al., 2001). These monkeys will continue to be monitored as they progress through menopause to identify changes in skeletal structure as they relate to changes in hormone status.

4. Reproduction

Consistent with a delay in skeletal maturation, reproductive maturation was also delayed in prepubescent monkeys exposed to CR early in life. The maturational increase in circulating testosterone levels was delayed by at least one year compared to CON monkeys (Roth et al., 1993). A similar finding was observed in CR rodents (Merry and Holehan, 1981) and undernourished humans (Bongaarts, 1980). It is unknown whether this delay in maturation indicates an early signal of increased lifespan.

With age, total and free testosterone decline in humans, a change causally linked to other age-associated changes, such as decreased fat mass and muscle strength, bone loss, and the development of central obesity (Tenover, 1997). This age-related decline in circulating testosterone levels has not been detected in the NIA colony from either cross-sectional or longitudinal measures. Schwartz and Kemnitz (1992) reported the existence of a trend toward lower testosterone levels in male rhesus monkeys over 20 years of age but due to the low number of older animals, the reported difference was not statistically significant. Kaler et al. (1986) reported similar mean 24-hour plasma testosterone levels between young and old monkeys but the pulsatile pattern of secretion differed between age groups. Because testosterone concentration varies widely amongst individuals and throughout the day, even controlled sampling time has not eliminated the high variability and thus has obscured potential age-related or diet effects on this hormone. Further studies are underway to obtain more frequent samples and a clearer understanding of how CR affects age changes in testosterone concentration in rhesus monkeys.

In females, the pattern of reproductive senescence in rhesus monkeys is similar to that in humans; however, relative to lifespan, changes in rhesus monkeys occur slightly later in life (Lane et al., 2001; Black and Lane, 2002). These hormonal changes leading to menopause in women are accompanied by changes in skin and body composition, loss of bone mass, cognitive impairment, and increased risk for cardiovascular disease in both humans and rhesus monkeys (Lindsay et al., 1996). These similarities make the rhesus monkey a valuable model for the study of menopause. Thus, it was of interest to determine if CR altered the age-related decline in reproductive function as has been shown in aging rats (Holehan and Merry, 1985).

Consistent with previous findings of hormonal changes in aging female monkeys, data from 40 female rhesus monkeys aged 7–27 years indicated a significant age-related decrease in serum estradiol, increase in follicle stimulating hormone (FSH) (Fig. 4), and a decrease in both number and length of menstrual cycles (Fig. 5). Progesterone and luteinizing hormone (LH) did not change with age (Fig. 4; Lane et al., 2001). Unlike rodent studies, these parameters were not altered by 6 years of 30% CR. This species difference may be due to a more moderate restriction in monkeys, 30% compared to 40% in rodents. Moreover, rodents have an estrus cycle of only 4–5 days compared to the 24–31 day menstrual cycle in rhesus monkeys and humans. In addition, Black et al. (unpublished data) have shown that short-term (4 months) 30% restriction did not alter ovulation in 8-year-old rhesus monkeys, indicating that nutrition in this species was adequate to support ovulatory function. This lack of a CR effect in female monkeys may benefit their reproductive success.

5. Glucoregulation

Considering their lower body weights and body fat, it is not surprising that CR monkeys are better able to regulate glucose than CON. Both rodent (Koizumi et al., 1989; Masoro et al., 1983; Reaven et al., 1983) and rhesus monkey

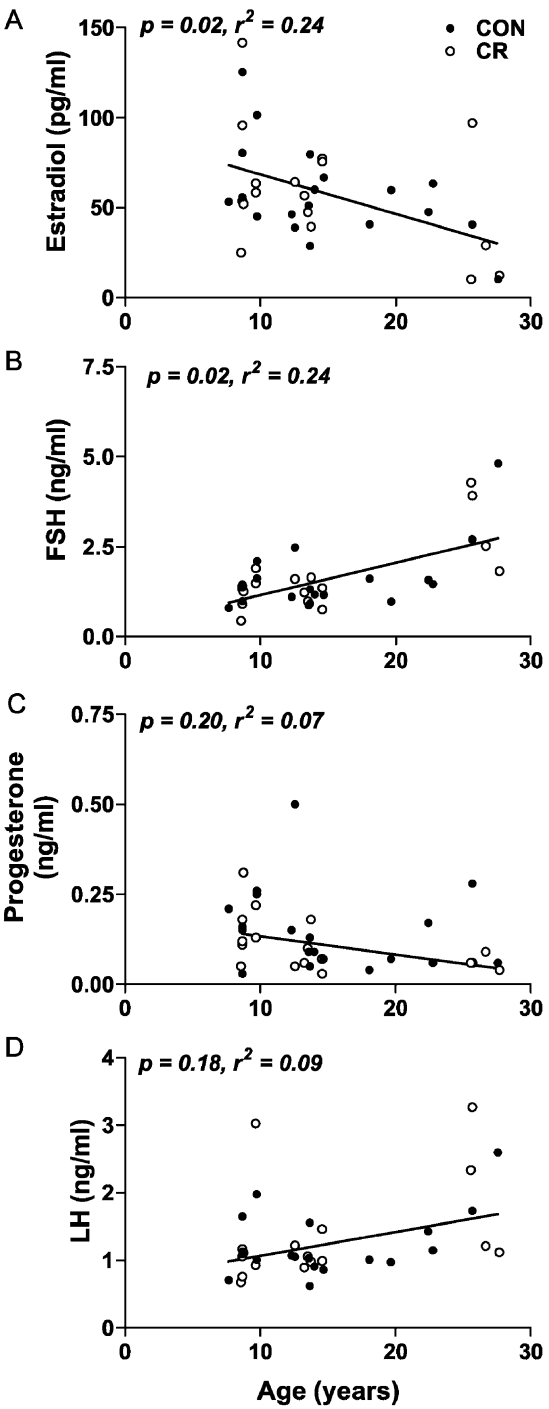

Fig. 4. Serum estradiol (A), follicle-stimulating hormone (FSH) (B), progesterone (C), and luteinizing hormone (D) concentrations in control (CON, $n = 21$) and 30% CR ($n = 19$) female rhesus monkeys. Each point represents biochemical data for individual monkeys at the corresponding age. Data were collected after 6 years of CR. Linear regression revealed significant effects of age on estradiol and FSH concentrations ($P = 0.02$). CR did not affect reproductive hormone concentrations. Reprinted from Lane et al. (2001).

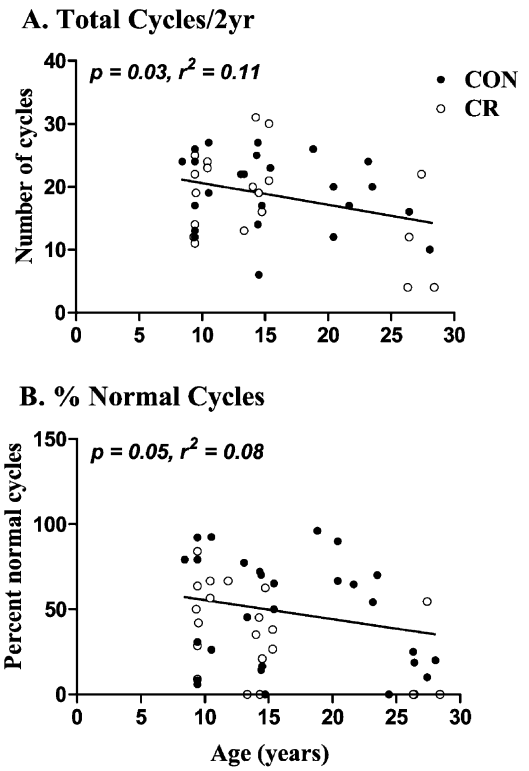

Fig. 5. Total number (A) and percent normal (24–31 d) (B) menstrual cycles in control (CON; $n = 21$) and 30% CR ($n = 19$) female rhesus monkeys over a 2 year period. Each point represents data for individual monkeys. Linear regression analysis revealed that both total number and percent normal menstrual cycles declined with age. CR did not affect menstrual cycling ($P > 0.05$). Reprinted from Lane et al. (2001).

data (Kemnitz et al., 1994; Lane et al., 1995b) support the conclusion that CR is effective at lowering fasting glucose and insulin levels. Monkeys in the NIA study required many months of CR before a reduction in fasting glucose was observed (Cutler et al., 1992); however, a subsequent report after 3 years of CR showed significantly reduced levels compared to CON (Lane et al., 1995b). This delayed onset of glucoregulatory adjustments with CR was consistent with another ongoing rhesus study at the University of Wisconsin (Kemnitz et al., 1994) but has not been reported in rodent studies. This difference may be due to differences in body composition, relative percentage of lifespan on CR, diet composition, or the severity of CR (Lane et al., 1995b).

In addition to lowering fasting glucose levels in CR rhesus monkeys, the maximum glucose level reached during an intravenous glucose tolerance test was lower compared to CON. The acute insulin response to a glucose load was also lower in CR monkeys (Lane et al., 1995b). These data agree with suggestions that CR increases insulin sensitivity and therefore could postpone the onset of type II diabetes.

The concomitant decrease in adiposity would certainly be a factor for the improved glucoregulatory function in CR monkeys; however, in the present study CR-induced differences occurred in relatively lean (<22% fat) monkeys suggesting a mechanism beyond changes in body composition. A concurrent study of short-term CR at the NIA showed that less than 1 year of CR also reduced fasting and peak insulin levels prior to any

changes in body composition (Lane et al., 1999b). Although the mechanism for this improved glucoregulation is unclear, these findings support Masoro et al. (1992) theories that CR may be altering basic mechanisms of glucose fuel use.

6. Cardiovascular disease

As the leading cause of death in the United States (American Heart Association, 2001), cardiovascular disease is clearly a major health concern. Diet and exercise are obvious interventions to reduce the risk of this disease. In the present study, both CON and CR monkeys were fed a diet that was low in total fat, saturated fat, and cholesterol; thus, both groups have generally low plasma cholesterol levels (105 ± 7 and 112 ± 6 mg/dl for adult CON and CR, respectively; Verdery et al., 1997). Verdery et al. (1997) also reported that CR was effective in lowering triglyceride levels in young and adult monkeys and increasing levels of the larger high density lipoprotein subfractions (HDL_{2b} and HDL_{1+2b}). Low levels of these HDL subfractions have been associated with increased cardiovascular disease in humans (Buring et al., 1992) and thus CR suggests a protective effect. This beneficial effect is presumably linked to changes in production and/or turnover of specific HDL components or subfractions which may be the result of changes in glucose homeostasis and body composition (Verdery et al., 1997).

An age-associated increase in blood pressure is another well-recognized risk factor for cardiovascular disease. Age-related differences in blood pressure have not been evident in the NIA monkeys; however, Lane et al. (1998) reported significantly lower blood pressure in females after 3 years on CR compared to CON. A reduction in blood pressure is associated with lower body weight in human studies (Adachi et al., 1996) and may be an important benefit of CR for the prevention of cardiovascular disease.

7. Metabolic parameters—energy balance, body temperature, and activity

Several hypotheses related to the mechanism for the biological effects of CR relate to reduced energy metabolism (Sacher, 1977; Harman, 1981); thus, it became important to characterize the effect of CR on rhesus monkeys in the NIA study as this intervention affected energy metabolism, body temperature, and activity.

Energy balance was determined as the difference between energy intake and energy loss, both fecal and 24-hour energy expenditure, in male rhesus monkeys subjected to CR for at least 4.5 years. Lane et al. (1995c) reported that despite approximately 30% lower caloric intake and body weight in the CR group, the amount of energy lost in the feces and fecal energy density were not different than that in age- and weight-matched controls (Table 1). Although energy expenditure was apparently lower in the CR group, the differences were not significant. Both CR and CON groups were determined to be in energy balance as net energy was not significantly different from zero. Overall results from these studies suggest that despite a significant reduction in caloric intake, the ability to extract energy from the diet was not altered by CR. These findings are in general agreement with rodent studies (McCarter et al., 1985; Duffy et al., 1989).

Rhesus monkeys on long-term (6 years) CR have reduced colonic body temperature (approximately 0.5 °C) compared to CON (Lane et al., 1996). This finding in monkeys agrees with previously reported CR rodent data (Weindruch and Walford, 1988; Duffy et al., 1989). This reduction in body temperature was consistent across an age range of 7–13 years and was apparent with a single time point measurement. To better characterize the effect of CR on body temperature, an additional group of monkeys was implanted with radiotelemetry devices under the skin to enable 24-hour monitoring while CR was being initiated. Serving as their own controls, these monkeys showed a gradual decrease in temperature as food intake was reduced. When the level of restriction reached 30%, body temperature was significantly ($p < 0.003$) lower than control levels and

Table 1
Energy intake and loss during long-term diet restriction in rhesus monkeys reprinted from Lane et al. (1995c)

	Group	Intake energy (kcal/day)[a]	% Diet restriction[b]	Fecal energy loss (kcal/day)[c]	Fecal energy density (kcal/day)[c]
Juvenile	CON	844 ± 25		133 ± 6	3.7 ± 2
	CR	574 ± 16#	31	112 ± 13	3.6 ± 7
Adult	CON	903 ± 16		103 ± 16	3.6 ± 3
	CR	667 ± 18#	26	116 ± 12	3.6 ± 1
Old	CON	480 ± 43*		98 ± 15	3.6 ± 1

*Significant correlation with chronological age, $r = 0.90$, $p < 0.001$. #Significant correlation with main effect of diet, $F(1, 19) = 86.5$, $p < 0.0001$.
[a] Each value represents the mean (±SEM) energy intake for monkeys in a diet group.
[b] Each value represents the average percentage diet restriction (reduction in caloric intake) for J and A group monkeys based on food consumption and dietary intake data.
[c] Each value represents the mean (±SEM) for monkeys in a diet group.

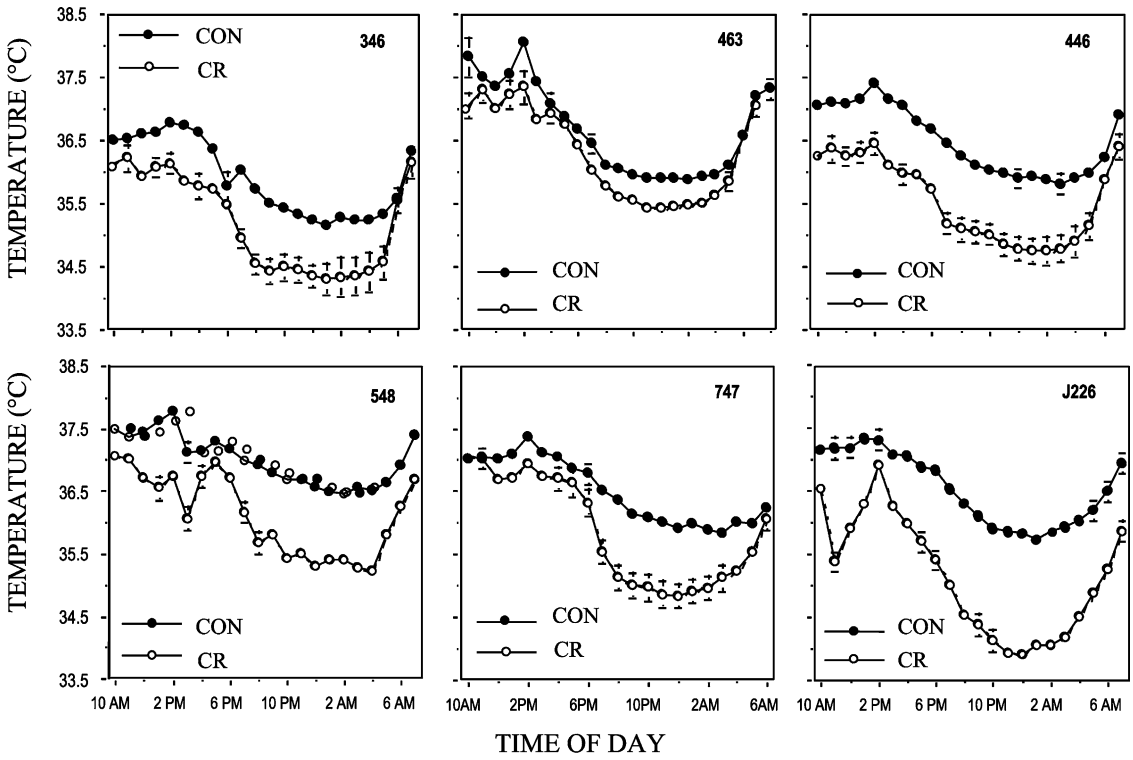

Fig. 6. Subcutaneous body temperature during CON feeding and 3 months at 30% CR for monkeys in a short-term study. Each point represents the mean (±SEM) hourly temperature measured continuously for 7 days. Error bars not shown are too small for graph scale. Reprinted from Lane et al. (1996).

circadian patterns were maintained (Fig. 6; Lane et al., 1996).

Although there is no direct evidence that reduced body temperature prevents disease or prolongs life, it is consistent with indirect findings in hibernating animals. When hibernation and thus the concomitant drop in body temperature was prevented in hamsters (Lyman et al., 1981) and reptiles (Saint Gironss, 1952), lifespan was shortened. These changes in body temperature occurring with CR may be one mechanism related to a metabolic shift to provide anti-aging effects. Interestingly, a recent report suggests that humans with lower body temperatures may have greater survival rates than those with higher temperatures (Roth et al., 2002).

Considering the changes in body weight and reduced temperature associated with CR, it was of interest to determine corresponding changes in locomotor activity and basic behavior patterns. Using ultrasonic motion detectors and videotape, behavior was monitored in males after 6 years on 30% CR. Weed et al. (1997) reported that daily activity and behavioral patterns were typical for captive housed primates over a 24-hour period (Line et al., 1989; Wolden-Hanson et al., 1993) and that CR did not adversely affect behavior in general. Among the adult males, CR monkeys displayed more pacing, gross movement, stereotypes, and were less passive than those in the control group (Weed et al., 1997). A subsequent study in the female cohort showed no generalized diet effect on activity although CR juveniles (6–8 years) were slightly less active than age-matched controls (Moscrip et al., 2000). Our findings in CR males of increased activity are consistent with data from rodent CR studies (Duffy et al., 1989, 1990; Goodrick et al., 1983) but possibly most importantly provide evidence that long-term CR did not negatively influence these behavior patterns.

8. Biomarkers

Due to the high cost and time necessary to evaluate putative anti-aging interventions, the development of biological markers to measure age-related change may be of great value to gerontologists. Although there is still considerable debate about what constitutes a biomarker of aging and how they should be validated, once their utility and validity are established, gerontological research will benefit greatly. Therefore, in addition to studies to determine the mechanism of CR, the NIA study has sought to identify candidate biomarkers that could be effectively altered by CR.

Human studies have shown that DHEA, an androgenic steroid, satisfies established criteria for candidate biomarkers of aging (Nakamura et al., 1994) that include cross-sectional (Orentreich et al., 1984; Ohasi et al., 1986) and longitudinal (Thomas et al., 1994; Orentreich et al., 1992) age-related decline, and maintenance

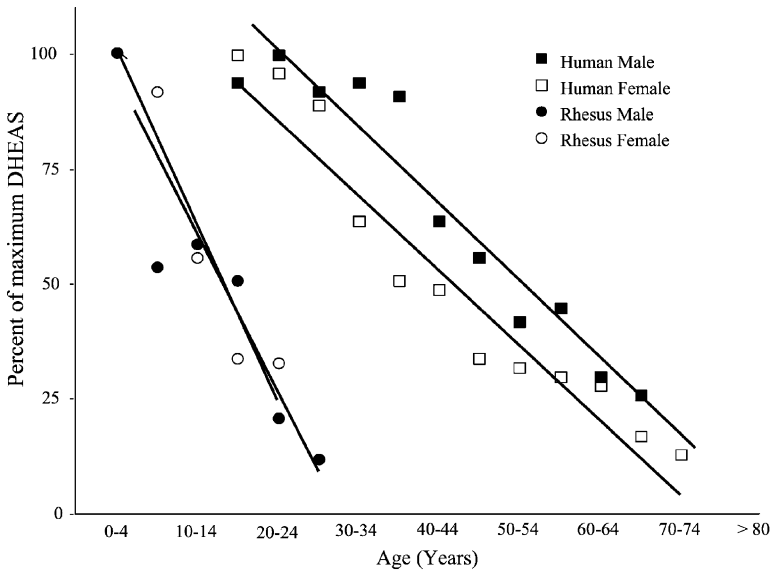

Fig. 7. Rate of serum DHEAS decline in humans (adapted from Orentreich et al., 1984) and rhesus monkeys. Each point represents the percentage reduction from maximal DHEAS levels in a given age group. Reprinted from Lane et al. (1997b).

of inter-individual differences over time (Thomas et al., 1994). DHEA and its sulfated form, DHEAS, are among the most abundant steroids in the body and have received attention as a possible hormonal intervention against the decrements of aging. Elevated serum levels of DHEAS have been related to a protective function against age-associated diseases, such as diabetes (Small et al., 1989), heart disease (Barrett-Connor et al., 1986), and cancer (Zumoff et al., 1981).

A recent large-scale survey of 792 laboratory-housed male and female rhesus monkeys reported an approximately 90% reduction in DHEAS from infancy through 3 years of age (Kemnitz et al., 2000). The magnitude of the decrease was greatest during the first few years of life followed by a more gradual decline, averaging 4.2% per year (Kemnitz et al., 2000). In humans, DHEAS peaks around 20 years of age and then decreases continuously thereafter in both men and women (Orentreich et al., 1984; Carlström et al., 1988). Although the slope of the decline clearly differs in these two species, the rate is about 2–2.5 times higher in rhesus monkeys compared to humans, a rate that is consistent with humans' approximately threefold greater lifespan (Fig. 7; Lane et al., 1997b).

It seemed possible that CR, an intervention widely known to delay age-related decline in function and increase lifespan in rodents, may prevent the age related decline in DHEA and DHEAS levels in rhesus monkeys. Roth et al. (1993) reported an age-related decrease in DHEA in males that was not affected by 2–3 years of CR (Roth et al., 1993). However, subsequent data following 3–6 years of CR showed that the post-maturational decline in serum DHEAS level was significantly attenuated in the CR group compared to CON (Fig. 8; Lane et al., 1997b). In the four years for which data were reported, DHEAS declined an average of 30% in adult male CON but only 3% in CR males during the same period. This finding provides evidence for the beneficial effects of CR in postponing the age-related decline in hormonal function.

Another potential biomarker of aging susceptible to the effects of CR is the hormone melatonin. Melatonin is secreted by the pineal gland and several other tissues, including the gastrointestinal tract, in a pulsatile pattern peaking in the early morning (Klein et al., 1992). Melatonin has been reported to improve sleep, increase longevity, lower blood pressure, strengthen the immune system, and act as an antioxidant (Chase and Gidal, 1997; Skene et al., 1999; Anisimov et al., 1998; Nishiyama et al., 2001; Reiter, 1995). With advancing age the pineal gland shrinks and melatonin secretion decreases and may be accompanied by a shift in the phase of its pulsatile release (Nishiyama et al., 2001; Kripke et al., 1998). It has been suggested that melatonin secretion from the gastrointestinal tract increases when calories are restricted; however, until recently there have been no controlled studies of the effect of CR on plasma melatonin in vivo.

Cross-sectional data from 52 control male and female rhesus monkeys in the NIA study confirmed findings in humans of an age-related decline in peak melatonin levels. Interestingly, an age-related decline was not evident in the monkeys that had been maintained on restriction for a 12-year period (Fig. 9; Roth et al., 2001). In fact, melatonin levels in old CR monkeys were significantly greater than that observed in the age-matched controls. Findings of sustained pineal function into old age in the CR monkeys in the NIA study are consistent with those in CR rats (Stokkan et al., 1991) and suggest beneficial effects of CR on slowing this aging process. Our finding that CR was not effective at

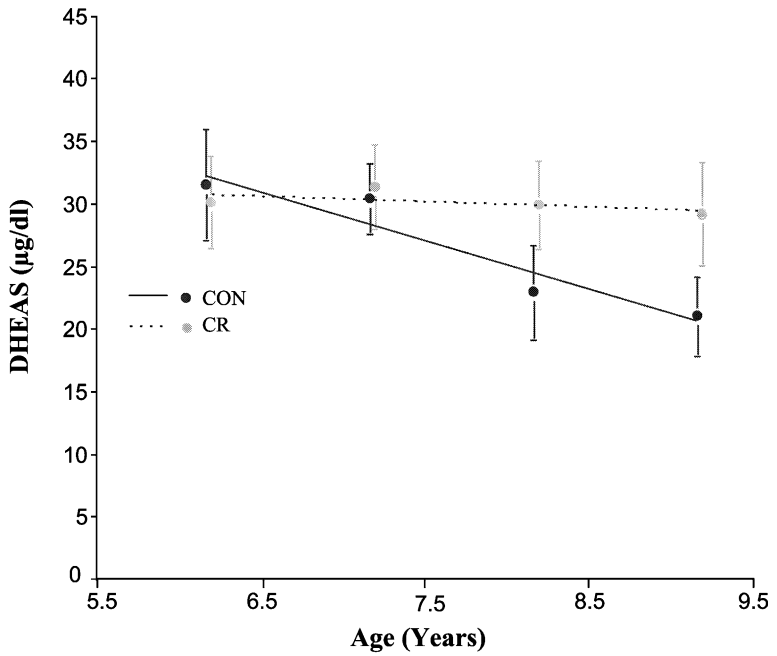

Fig. 8. CR slows the rate of decline in serum DHEAS. Each point represents the mean (± SEM) DHEAS level at a given age. Ages represent the average (± 0.3 year) age of young adult male rhesus monkeys for years 3–6 of the longitudinal study. Slopes indicating the rate of decline were calculated from the regressions shown for each monkey group (CON = −3.9 µg/dl per year and CR = −0.4 µg/dl per year). The rate of change was significantly slower in CR monkeys ($P < 0.005$). Reprinted from Lane et al. (1997b).

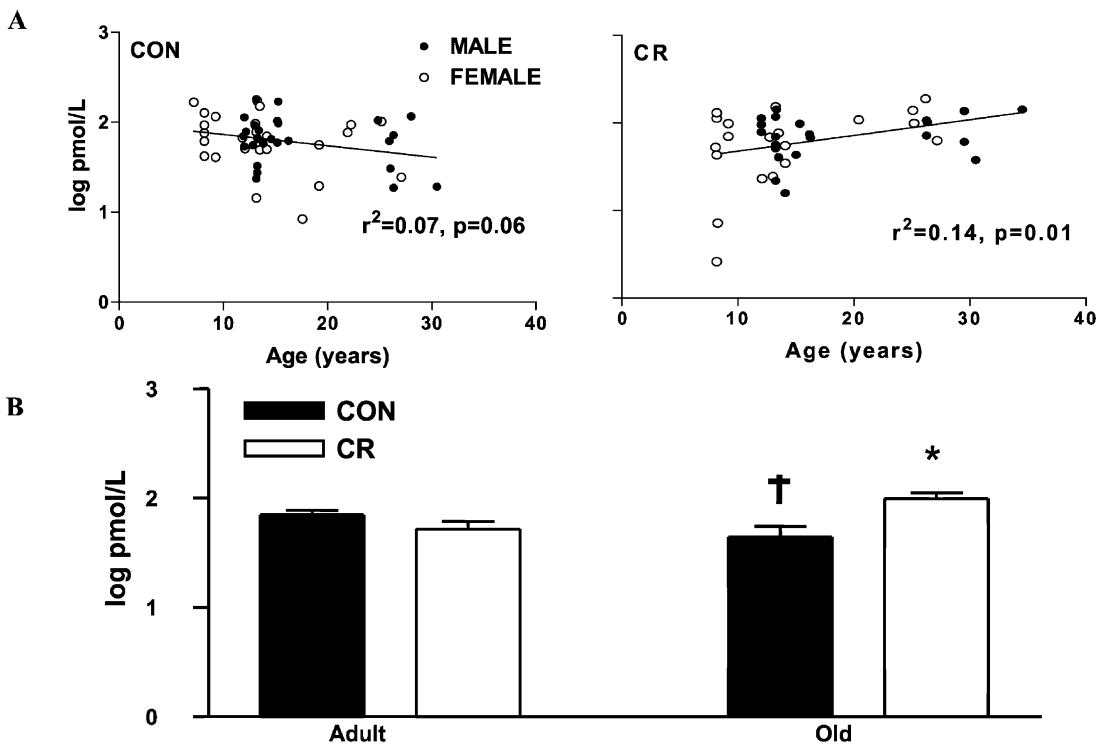

Fig. 9. The effect of aging and CR on plasma melatonin in rhesus monkeys. Panel A summarizes the relationship between age and melatonin levels (log transformed) for CON and CR rhesus monkeys. Panel B represents the mean (± SEM) melatonin levels in adult and old monkeys. The age × diet interaction is significant ($p < 0.04$) by two-way analysis of variance. † Analysis of the simple main effect of age indicated a significant decrease in the CON group ($P < 0.04$), but not in the CR group. *Analysis of the simple main effect of diet indicated a significant effect in the old group ($P < 0.01$), but not in the young group. Reprinted from Roth et al. (2001).

altering melatonin secretion in the adult animals was not surprising, as they likely have not experienced a significant age-related decline. Similarly, adult animals in the University of Wisconsin study do not show signs of a CR effect on melatonin level (Ramsey et al., 2000). Diet differences may emerge as the NIA monkeys are monitored into old age.

9. Morbidity, mortality, and future functional studies

With an average lifespan of 25 years and a maximum of 40 years, studies of longevity in rhesus monkeys are challenging to conduct. Thus it is difficult to formulate a strategy for demonstrating the effectiveness of this nutritional intervention that is both robust and reliable. Criteria were established at a workshop held in 1999 to create a program that could evaluate the effectiveness of putative anti-aging interventions. Warner et al. (2000) reported from this meeting the need for endpoints in addition to survival data. Although a demonstration of increased mean and maximum lifespan is necessary, there is also a requirement that any intervention preserve health and function. Effective anti-aging interventions should result in decreasing the incidence and delaying the age of onset of characteristic age-related diseases and pathology. In addition, there must be maintenance of cellular, organ, physiologic, and behavioral function into old age. By using criteria in these three main categories of mortality, morbidity, and function, the NIA hopes to clearly establish that CR retarded the rate of aging in rhesus monkeys.

With a current survival of 81% after 15 years of study, it is clearly not possible to evaluate mortality conclusively at this time. Moreover, it may be another decade before this is feasible. Preliminary data does show that the number of monkeys dying from age-related disease processes is greater in the control group (13 CON vs. 7 CR). Although mortality data is encouraging, it is unclear whether there will be sufficient power with this number of animals to statistically demonstrate longevity benefits of CR.

A second goal of showing a decreased incidence of morbid events also shows promise. Thus far, there have been fewer incidence of chronic disease, including neoplasia, cardiovascular disease, diabetes, endometriosis, fibrosis, amyloidosis, ulcers, cataracts, and kidney failure in CR monkeys compared to CON. This preliminary finding suggests that CR may provide protection against certain disease states that may prove beneficial for longevity. Interestingly, CON and CR monkeys appear to be equally susceptible to acute conditions such as inflammation, infection, hernia, anemia, and dental problems.

Another current goal in the NIA study is to provide convincing evidence that CR contributes to the maintenance of function in middle-aged monkeys as they progress into old age. Although much of the history of this study has focused on assays of molecular markers and mechanisms of action, efforts are shifting toward a strategy to design and implement experiments that evaluate function of physiological systems in a comprehensive manner.

This strategy for evaluating function was designed to employ reliable measures in the least invasive and safest possible manner. Because the number of old animals remaining in the study is low, only the monkeys originally designated as juveniles and adults will be used for these assays. Table 2 lists the current inventory of animals. The first round of assays will be conducted over the next three years on groups of 12–15 CON and CR monkeys. The second, follow-up, round of identical assays will occur 3–5 years after the first assay in 8–12 CON and CR monkeys. These estimates of sample size account for deaths that will inevitably occur over the next several years yet are large enough that group differences should be apparent by statistical evaluation.

The proposed functional assays will provide both a thorough and relevant assessment on the effectiveness of CR at delaying the onset of age-related diseases. We recognize that many areas of physiological interest are not included. Although it would be ideal to include additional tests, studies are limited because of their potential invasiveness and feasibility. For example, anesthesia requirements prevent tests of pulmonary function in which accurate results require an awake and compliant subject.

Many of our proposed projects will supplement current knowledge of the effects of CR on aging but will expand the database to include long-term effects in old animals. For example, we have shown that CR monkeys have a more efficient insulin response to a glucose load than CON animals and thus presumably have a decreased risk for diabetes. We do not, however, know if this adaptation is maintained throughout life. Similarly, measures of blood lipids and triglycerides and their potential to decrease the risk for cardiovascular disease have been established early in the NIA study, but we hope to discern the potential lifelong value of CR on these measures. To further evaluate cardiovascular disease risk, measurements of pulse wave velocity, a noninvasive measure of vascular stiffness known to increase with age, will also be conducted.

Other tests of organ systems include the ability of the liver to detoxify an innocuous drug, creatinine clearance by

Table 2
Current inventory of NIA Rhesus monkeys

Sex	Age group	CON (n)	Age range (years)	CR (n)	Age range (years)
Males	Adult	19	16–20	15	16–20
	Old	7	29–34	6	30–38
Females	Adult	19	11–23	16	18–24
	Old	3	26–29	2	30–31

the kidney, structural changes of the brain using magnetic resonance imaging (MRI), and neurochemical changes of the brain using positron emission tomography (PET). Tests of the ear will determine cochlear and neural function; accommodation, cataracts, and macular degeneration will be evaluated for the eyes; periodontal disease and inflammatory responses will be tested in the mouth. Tests of T-cell distribution and response will be used to evaluate immune function. Skeletal health will be monitored with yearly DXA scans and assessment of eight joint sites for osteoarthritis.

Reproductive function will be evaluated in females by continued daily monitoring of menstrual cycles. In addition, reproductive hormones such as estradiol, progesterone, FSH, LH, and inhibin A and B will be assessed during two menstrual cycles. Lastly, because ovarian function has been shown to decline with age and may be related to follicle depletion, decreased oocyte number, or poor quality of oocytes, a clomiphene citrate challenge will test ovarian reserve, or the pool of primordial follicles in the ovary. Clomiphene is a selective estrogen receptor modulator used for the induction of ovulation and predictive of diminished ovarian reserve. In males, sperm count, quality, and ability to fertilize will be measured.

Behavior testing will make up a large component of our future studies. The NIA is currently in the process of evaluating learning and memory ability in tasks of object discrimination and reversal learning. In addition, locomotor activity will be re-evaluated and monkeys will be tested for gross and fine motor coordination.

10. Conclusion

CR remains the only intervention shown consistently to extend lifespan and slow the rate of aging in short-lived animals. Rhesus monkeys exhibit many beneficial changes associated with CR that are similar to that observed in rodents such as decreased fat, improved glucoregulatory function, and decreased incidence of risk factors for cardiovascular disease and diabetes. Although preliminary evidence suggests that rhesus monkeys will have similar life-extending benefits from this intervention, it will be several years before lifespan data can be verified statistically. Thus, in addition to morbidity and mortality, our proposed functional assays will provide evidence for the effectiveness of CR in maintaining not only an increased lifespan but also a healthy one.

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