

Calorie Restriction in Nonhuman Primates: Effects on Diabetes and Cardiovascular Disease Risk

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The effects of calorie restriction (CR) on life span, disease, and aging in physiological systems have been documented extensively in rodent models. However, whether CR has similar effects in longer-lived species more closely related to humans remains unknown. Studies of CR and aging using nonhuman primates (rhesus monkeys) have been ongoing for several years at the National Institute on Aging and the University of Wisconsin-Madison. The majority of data published from these studies are consistent with the extensive findings reported in rodents. For example, monkeys on CR weigh less and have less body fat. Monkeys on CR also exhibit lower body temperature, fasting blood glucose and insulin, and serum lipids. In addition, insulin sensitivity is increased in monkeys on CR. Recent efforts in the NIA study have focused on the effect of this intervention on risk factors for various age-related diseases, in particular for diabetes and cardiovascular disease. We have shown that monkeys on CR have lower blood pressure, reduced body fat, and a reduced trunk:leg fat ratio. Also, monkeys on CR have reduced triglycerides and cholesterol and have increased levels of HDL_{2B}. Low levels of this HDL subfraction have been associated with increased cardiovascular disease in humans. In short-term studies, older (>18 years) monkeys on CR exhibit reductions in insulin and triglycerides before changes in body composition and fat distribution became evident. These and other findings have suggested that CR might have beneficial effects on certain disease risk factors independent of reductions in body weight or prevention of obesity.

Key Words: rhesus monkeys; aging; biomarkers; sulfated dehydroepiandrosterone (DHEAS).

The overwhelming majority of research on the calorie restriction (CR) paradigm has been conducted in short-lived species, primarily laboratory rodents. This nutritional intervention is widely accepted as the only nongenetic method for extending life span and altering physiological processes related to aging in short-lived mammals. The effects of CR on life span, disease, and the molecular and physiological aspects of aging in rodents and other short-lived species have been well-described and reviewed in several publications (Fishbein, 1991; Hart *et al.*, 1995; Weindruch and Walford, 1988; Yu,

1994). Until recently, however, little was known regarding effects of CR in long-lived mammals more closely related to humans.

In 1987, the National Institute on Aging (NIA) began the first study of CR and aging in nonhuman primates utilizing rhesus monkeys of various ages (Ingram *et al.*, 1990). A subsequent study of adult onset CR was begun at the University of Wisconsin-Madison (UW), in 1989 (Kemnitz *et al.*, 1993). These studies involve approximately equal numbers of

TABLE 1
Summary of Findings from the NIA Study of Calorie Restriction in Nonhuman Primates

Finding	Reference	Agrees with rodent data
Growth and development		
↓ body weight	19–21	yes
↓ fat and lean mass	21,37	yes
↓ trunk:leg fat	8	—
time to sexual maturation	32	yes
time to skeletal maturation	19	yes
Glucoregulation		
↓ fasting glucose/insulin	20	yes
↑ insulin sensitivity	8	—
Metabolism		
↓ metab. rate (short-term)	22	yes
↓ metab. rate (long-term)	21	yes
↓ body temperature	22	yes
↓ or locomotion	43	yes
Serum lipids		
↓ triglycerides	38	yes
↑ HDL 2B	38	—
Other		
↓ IGF-1/growth hormone	7	yes
↓ IL-6	19	yes
↑ testosterone	unpublished	—
↑ estradiol, LH, FSH, Prog.	unpublished	—
↓ wound closure rate	33	yes
↓ clonal proliferation	31	yes/?
↓ β-gal senescent cells	31	?
↓ rate of decline in DHEAS	23	—
↓ lymphocyte number	40	yes
↓ lymphocyte calcium response	10	no

Note. ↓ = decreased, ↑ = increased, ↓ no effect or change, and —, not reported in rodents.

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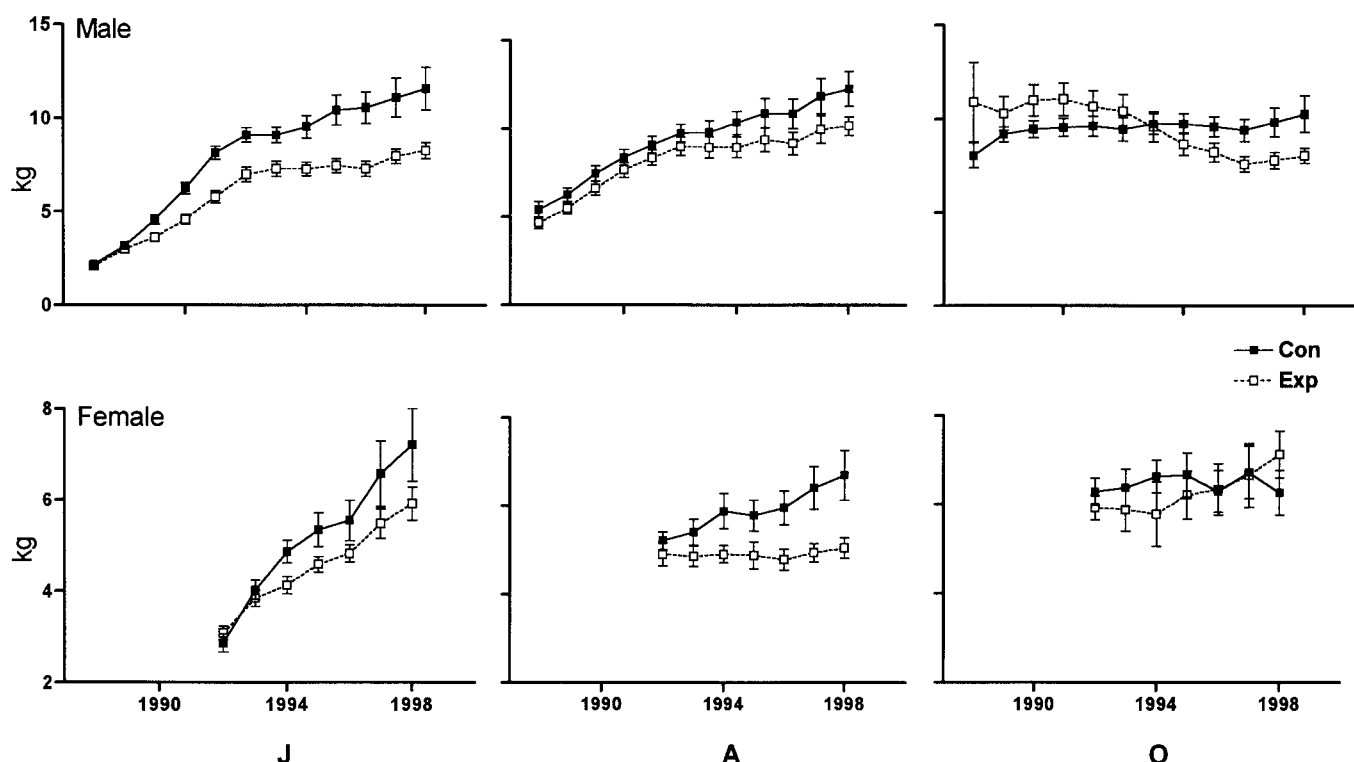


FIG. 1. Effect of calorie restriction on body weight for male (upper) and female (lower) rhesus monkeys. Each point represents the mean (\pm SEM) of annual body weight for monkeys in that group. The main effect of calorie restriction (analysis of variance, repeated measures) was significant for both genders (p values < 0.05).

control and CR subjects and were designed as longitudinal studies assessing the effects of CR on aging, disease, and longevity. Other studies of reduced calorie intake similar to the NIA and UW studies are underway at the Wake Forest School of Medicine (Cefalu *et al.*, 1997) and the University of Maryland (Bodkin *et al.*, 1995). In contrast to the NIA and UW studies, these investigations are more limited in their scope, as they focus mostly on cardiovascular disease and diabetes end-points. More details on both the UW (Weindruch *et al.*, 1998) and Wake Forest (Cefalu *et al.*, 1998) studies are presented elsewhere in these proceedings and the remainder of the present manuscript will focus on findings from the NIA study.

MATERIALS AND METHODS

In our laboratory, we are studying the effects of CR on aging in about 200 rhesus monkeys with an age distribution encompassing much of the rhesus life span. We initiated CR in monkeys of 3 different ages: Group J (1–2 years), Group A (3–5 years), and Group O (>15 years). All monkeys except one older male were born in captivity and had known dates of birth. Housing and husbandry practices have been described in previous publications (Ingram *et al.*, 1990; Lane *et al.*, 1992, 1995a).

All monkeys consume the same chow diet, which is supplemented with additional vitamins and minerals to guard against malnutrition in the CR groups. Monkeys are fed 2 meals per day at 0700 and 1400 h. About half the

monkeys serve as controls and are fed approximately *ad libitum* (AL), based on National Research Guidelines for nonhuman primates (National Research Council, 1978). Regular studies of food consumption have shown that control monkeys consistently eat at a level of intake that approximates AL feeding. Monkeys on CR receive 30% less food than the age- and body weight-matched controls. The diet consists of 72% crude carbohydrate, 15% crude protein, 5% crude fat, 8% fiber (by weight) and 3.77 cal/gram. Because all monkeys are fed the same chow, the feeding regimen results in a reduction in total caloric intake and not alteration of a specific dietary component.

Establishing a Primate CR Model

Initially, our studies at the NIA focused on establishing a nonhuman-primate model of CR, on determining if this nutritional intervention could be conducted safely in longer-lived species, and on whether many of the effects of CR reported in rodents also occurred in primates. Several reports have confirmed that rhesus monkeys on CR diets exhibit many effects similar to those reported in rodents. For example, we reported that monkeys on CR weigh less, have less fat, and have reduced lean body mass compared to controls (Lane *et al.*, 1995a and b; Tilmont *et al.*, 1996). In addition, CR delayed both sexual (Roth *et al.*, 1993) and skeletal (Lane *et al.*, 1995a) maturation in young male monkeys, as indexed by puberty-associated increases in serum testosterone, biochemical measures related to bone growth, and dual energy x-ray absorptiometry (DXA) measurements of bone mineral content in the skeleton. We have also reported that CR reduces rectal temperature measured in anesthetized monkeys after an overnight fast and by utilizing 24-h recording of body temperature via radio-telemetry implants (Lane *et al.*, 1996). Studies of glucoregulation, which will be discussed in greater detail below, showed that monkeys on CR exhibit

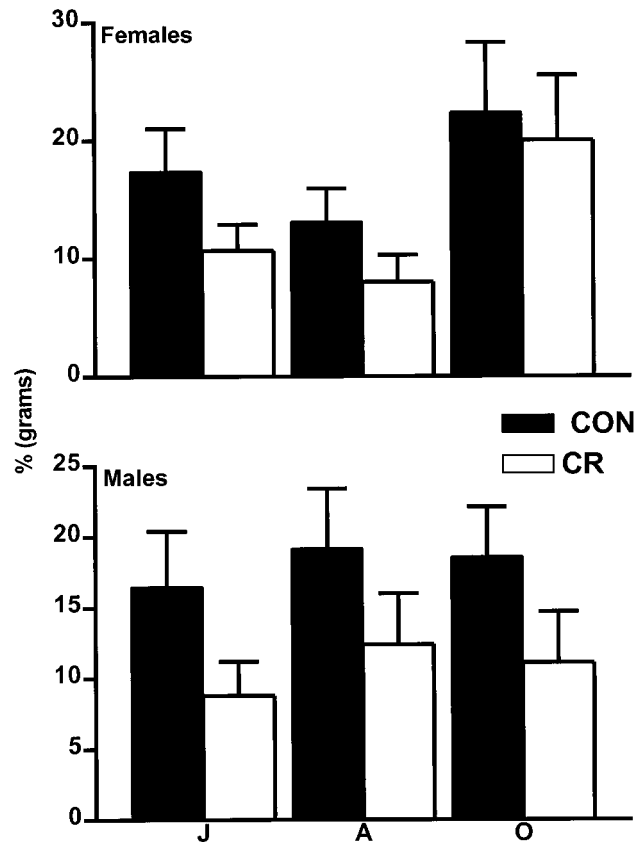


FIG. 2. Calorie restriction reduces percent body fat. Each bar represents the mean \pm SEM) percent body fat determined by dual energy x-ray absorptiometry after 6 (females) or 11 (males) years on CR. The effect of CR on reducing percent fat was significant for both genders (p values < 0.05).

changes similar to those reported in rodents (Lane *et al.*, 1995b). Further, studies of energy expenditure, using indirect calorimetry and isotopic dilution, have shown that, as reported in rodents, rhesus monkeys on CR exhibit a transient reduction in energy expenditure (Lane *et al.*, 1996), which is not maintained over the long term (Lane *et al.*, 1995c). Table 1 provides a summary of findings from the NIA study and compares many of the findings from rhesus-monkey studies to those reported in rodents.

RESULTS

Clearly, findings from our studies of rhesus monkeys on CR are in general agreement with published rodent findings (for review see Lane *et al.*, 1997a). It will be several more years before survival data are available to permit determination of whether CR has extended life span in monkeys. Nonetheless, extensive agreement with rodent studies, in which life span is clearly extended by CR, suggests that the effects of CR, possibly including life-span extension, may be universal among species. Having established a nonhuman primate model of CR, more recent work in the laboratory has focused on possible metabolic mechanisms of CR and effects of CR on risk factors for diabetes and cardiovascular disease. The remainder of this

paper focuses on our studies relevant to diabetes and cardiovascular risk.

Diabetes and Cardiovascular Risk

Obesity or central (abdominal) obesity is associated with hyperinsulinemia, insulin resistance, and other disease risk factors in humans.

The effects of CR on reducing body weight and fat in rodents are well established (for review, see Weindruch and Walford, 1988 and Yu, 1994). In addition, visceral (abdominal) fat is reduced in rodents on CR (Barzilai *et al.*, 1998). All of the studies of CR in rhesus monkeys have published findings relating to changes in body composition (for review see Lane *et al.*, 1997a). Figure 1 summarizes body weight data for over 11 years in male and 6 years in female rhesus at the NIA. It can be seen that CR not only reduced body weight, but that the rate of body weight gain was slowed in younger growing monkeys. Statistical analyses confirmed that CR significantly reduced body weight in both genders (p values < 0.05).

We have examined percent body fat in our colony utilizing

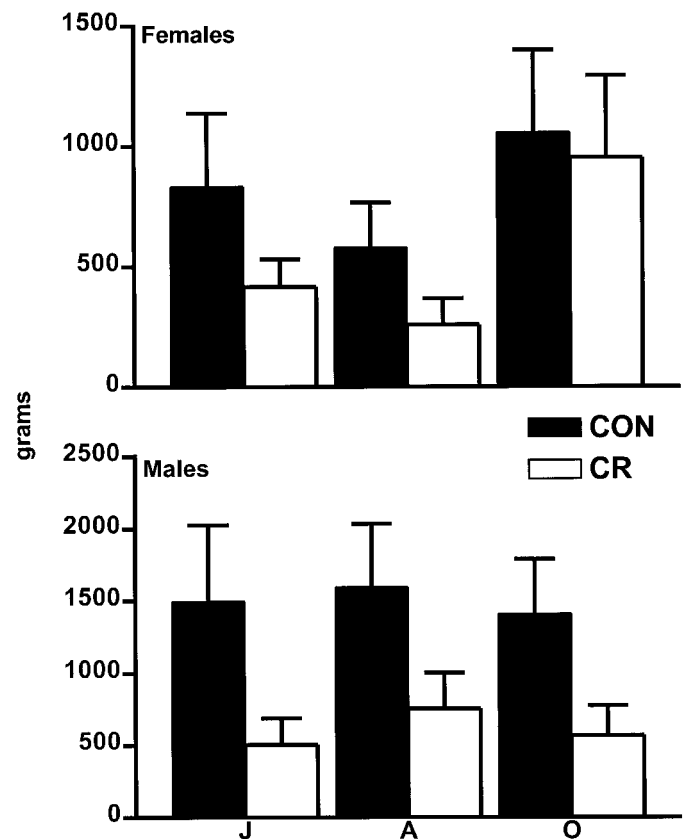


FIG. 3. Calorie restriction reduces abdominal (trunk) fat. Each bar represents the mean (\pm SEM) amount of trunk fat determined by dual energy x-ray absorptiometry after 6 (females) or 11 (males) years on CR. The effect of CR on reducing trunk fat was significant for both genders (p values < 0.05).

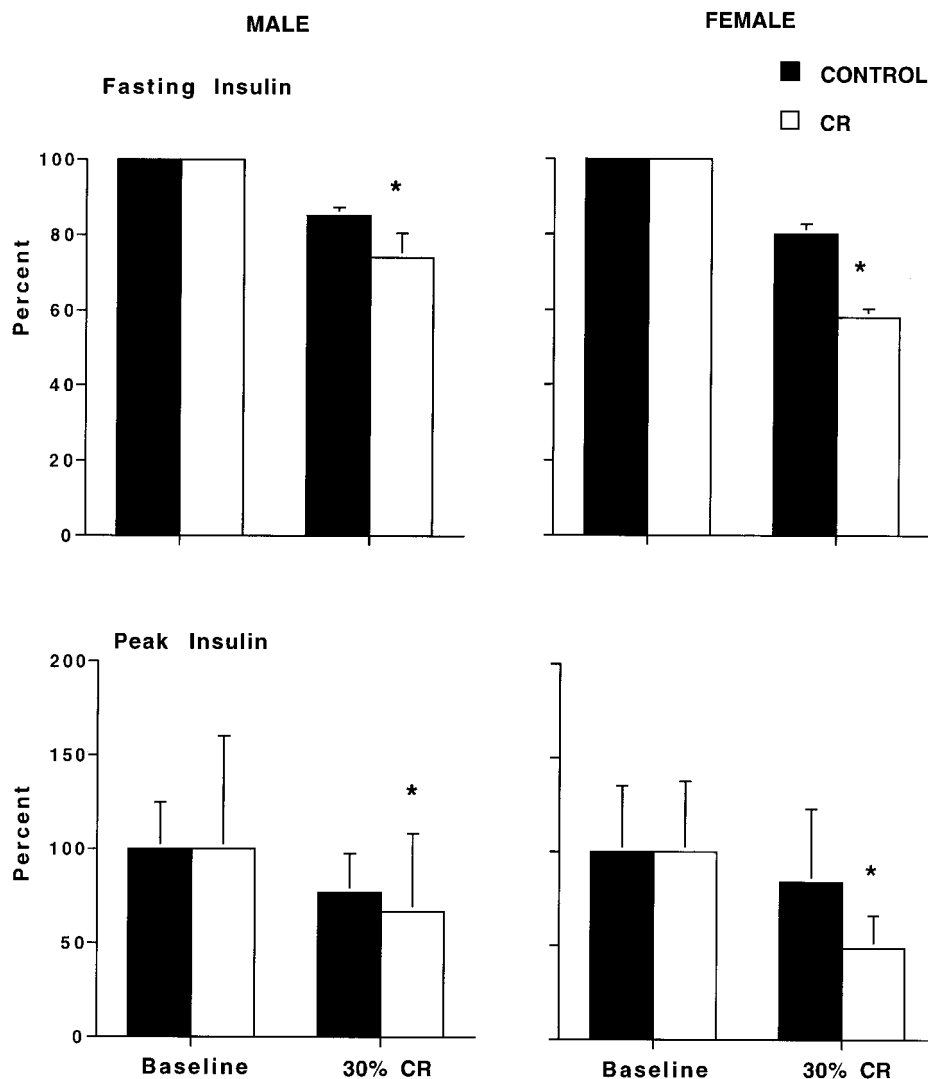


FIG. 4. Insulin responses during short-term calorie restriction in male and female rhesus monkeys. Each bar represents the fasting or peak insulin level (relative to the average baseline level). Baseline levels were measured during AL intake and are compared to similar data collected when CR monkeys were consuming 30% less food than controls. * Both fasting and peak insulin levels were significantly reduced at 30%, compared to AL feeding in CR, but not control monkeys. (Reprinted with permission from Lane *et al.*, 1998; *J. Anti-aging Med.*)

isotopic dilution (Lane *et al.*, 1995c) and DXA (Tilmont *et al.*, 1996). Both methods show that percent body fat is reduced in male CR monkeys when compared to controls. Figures 2 and 3 summarize our most recent data on body fat and fat distribution. At the time these data were collected male monkeys had been on CR for about 11 years and females for nearly 6 years. Figure 2 shows clearly that percent body fat is reduced in both male and female rhesus monkeys on CR (p values < 0.05). DXA data can also be analyzed to provide information on regional body composition. The validity of DXA for such regional analyses has been established in humans (Ley *et al.*, 1992; Svendsen *et al.*, 1993a,b). Figure 3 shows the amount of trunk fat determined by DXA in male and female monkeys.

Trunk fat was significantly reduced in CR monkeys, compared to controls (p values < 0.05), suggesting a reduction in abdominal fat.

Our findings that rhesus monkeys on CR have less body fat are in agreement with studies in rodents and in rhesus monkeys (Hansen and Bodkin, 1993; Kemnitz *et al.*, 1994). Further, the group at the University of Maryland have shown that the weight stabilization protocol (resulting in about 35% CR) prevents obesity and many of the complications associated with development of diabetes (Bodkin *et al.*, 1995; Hansen and Bodkin, 1993). The fact that CR also reduced trunk (abdominal) fat in our monkeys suggests a more favorable distribution of body fat away from the central or abdominal region. The

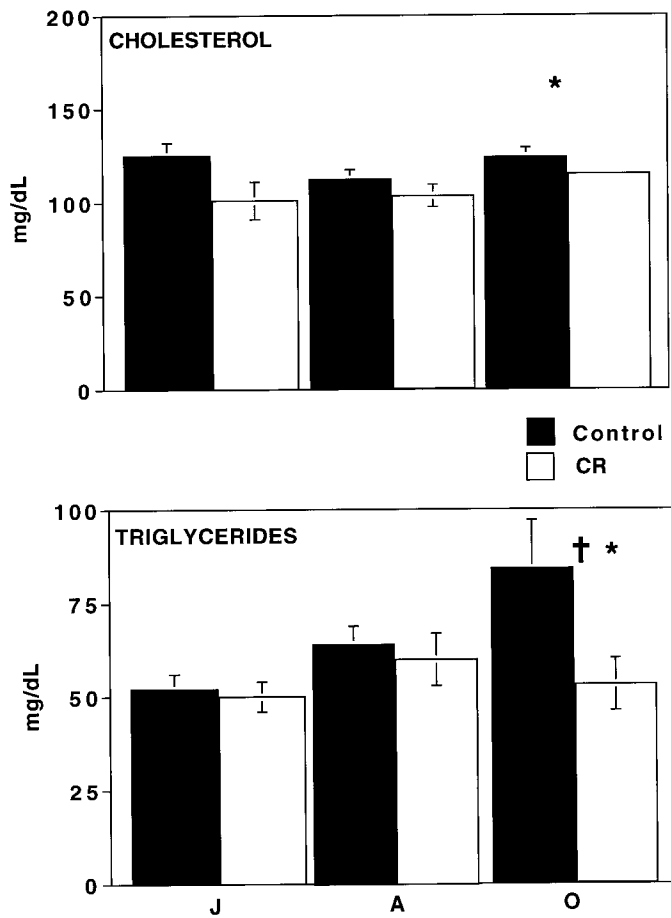


FIG. 5. Calorie restriction reduced total serum cholesterol and triglycerides in female rhesus monkeys. Each bar represents the average \pm SEM of total serum cholesterol or triglyceride level for monkeys in each group. There were 53 monkeys in 3 age groups, J (9 CON, 9CR), A (12 CON, 11 CR), and O (7 CON, 5 CR). *Triglycerides increased significantly with age and the * CR effect on lowering serum lipids was significant by analysis of variance (p values < 0.05). (Reprinted with permission from Lane *et al.*, 1998, *J. Anti-aging Med.*)

effects of CR on body composition suggest that disease risk associated with obesity/central obesity will be favorably impacted by this nutritional intervention.

Altered glucoregulation represents another major risk factor that is affected by CR. Several studies in rodents (Kalant *et al.*, 1988; Masoro *et al.*, 1989, 1992) and in rhesus monkeys (Bodkin, 1995; Hansen and Bodkin, 1993; Kemnitz *et al.*, 1994; Lane *et al.*, 1995b) have shown that CR favorably alters several glucoregulatory endpoints. For example, we have shown that after about 3 years on CR, male monkeys exhibit significant reductions in fasting glucose and insulin levels, as compared to controls (Lane *et al.*, 1995b). We also have studied the response to an intravenous glucose tolerance test in male monkeys on CR. Calorie restriction lowered peak glucose and insulin values and attenuated both the acute and second-phase insulin responses (Lane *et al.*, 1995b). In a subsequent

study, we demonstrated that CR improved insulin sensitivity in the same group of younger, lean male rhesus (DeAngelis *et al.*, 1998).

We have utilized a short-term CR paradigm to determine the temporal nature of changes in glucoregulation in this model. In these studies, we assessed body composition and glucoregulation during adaptation to the CR paradigm. Measurements were collected at baseline (AL) and following subsequent 10% reductions in food intake instituted gradually (10% per month) over a 3-month period. In both young female (2–3 years; Lucia *et al.*, 1996) and old male (>18 years; DeAngelis *et al.*, 1998) rhesus monkeys, we showed that CR reduced fasting and peak insulin during restriction to 30% of AL intake (Fig. 4). CR also attenuated the acute and second-phase insulin responses in these studies (data not shown). Interestingly, glucose-related endpoints such as fasting and peak glucose and glucose tolerance did not change during the same period. Moreover, DXA studies of body composition showed that there was little (female) or no (males) effect on body weight or percent body fat during this short-term study. These preliminary studies suggested that CR might have significant effects on glucoregulation, particularly insulin parameters, independent of changes in body composition.

DISCUSSION

Studies from our group and others show that the effects of CR on glucoregulation are likely to result in prevention or delayed onset of diabetes and clinical manifestations associated with hyperinsulinemia. Also, short-term studies suggest that insulin responsiveness and action are acutely sensitive to changes in food intake, highlighting the potential importance of insulin metabolism in the CR model. Finally, our findings suggest that the effects of CR on insulin secretion and action may, to some degree, be independent of changes in body composition.

We have also investigated the effects of CR on serum lipids in young, lean monkeys. A report by Verdery *et al.* (1997) showed that CR lowered serum triglyceride levels and increased levels of the HDL subfraction, HDL_{2B}, in young adult male rhesus monkeys. Low levels of HDL_{2B} have been associated with increased cardiovascular disease in humans (Buring *et al.*, 1992). Studies of lipids in female monkeys have so far been limited to total cholesterol and triglyceride levels. Figure 5 shows that CR lowered total serum cholesterol and triglycerides in female rhesus monkeys and prevented the age-related increase in triglycerides seen in controls (Lane *et al.*, 1999). We have continued to follow serum triglycerides on a yearly basis and have observed that levels remain significantly lower in both male and female rhesus on CR, compared to controls (unpublished data).

We have not noted an age-associated increase in hypertension in either male or female rhesus monkeys in our colony. Nonetheless, studies related to hypertension as a risk factor for

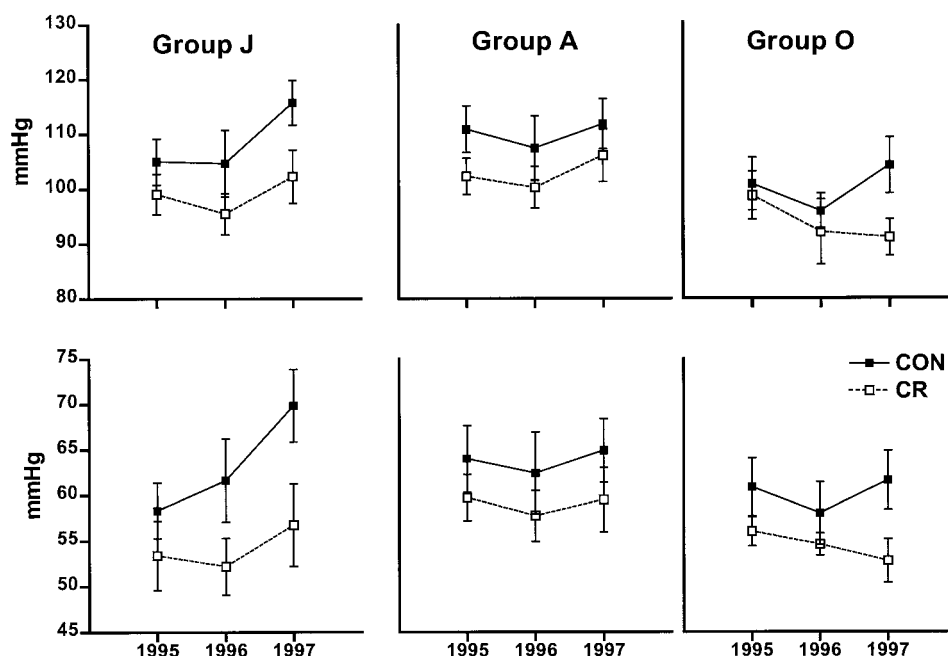


FIG. 6. Each point represents the average \pm SEM annual systolic or diastolic blood pressure for female monkeys in each age or diet group. There were 53 monkeys in 3 age groups, J (9 CON, 9CR), A (12 CON, 11 CR), and O (7 CON, 5 CR). *The effect of CR on reducing blood pressure was significant by analysis of variance (p value < 0.05). (Reprinted with permission from Lane *et al.*, 1998, *Journal of Anti-aging Med.*)

cardiovascular disease have yielded interesting findings regarding the effects of CR. For example, CR significantly lowered blood pressure (Fig. 6) in female rhesus (Lane *et al.*, 1999), and males on CR consistently exhibit reductions in both systolic and diastolic blood pressure. However, the effect of CR on blood pressure in males does not reach statistical significance.

It is unknown if serum levels of dehydroepiandrosterone (DHEA) and its sulfated form (DHEAS) play a meaningful role in aging or disease. However, these hormones decline markedly with age, and it has been suggested that elevated levels of these steroids may be associated with protection against several diseases such as diabetes (Small *et al.*, 1989), cardiovascular disease (Barrett-Connor *et al.*, 1986), and cancer (Zumoff *et al.*, 1981). We have shown that as reported in humans, serum DHEAS levels decline markedly with age in rhesus monkeys (Lane *et al.*, 1997b). We conducted a longitudinal study to determine if CR altered the rate of decline of serum DHEAS levels in young adult male monkeys (Fig. 7). As expected, serum DHEAS declined significantly over the course of the 4-year study. Interestingly, the rate of decline was significantly less in CR monkeys when compared to controls of the same age ($t = 4.2$, $p < 0.002$). The relationship of this effect of CR on DHEAS levels to aging and disease has not been fully elucidated. However, this finding suggests that CR might retard postmaturation aging as indicated by the slowed decline in DHEAS levels.

The existing data relevant to diabetes and cardiovascular disease suggest a significant reduction in risk potential in monkeys on CR. Our findings that CR favorably alters these

risk factors are unique in that total intake, and not the proportion of specific dietary components, has been altered and that these positive effects were seen in CR monkeys compared to lean, normal-weight controls. Most human and nonhuman pri-

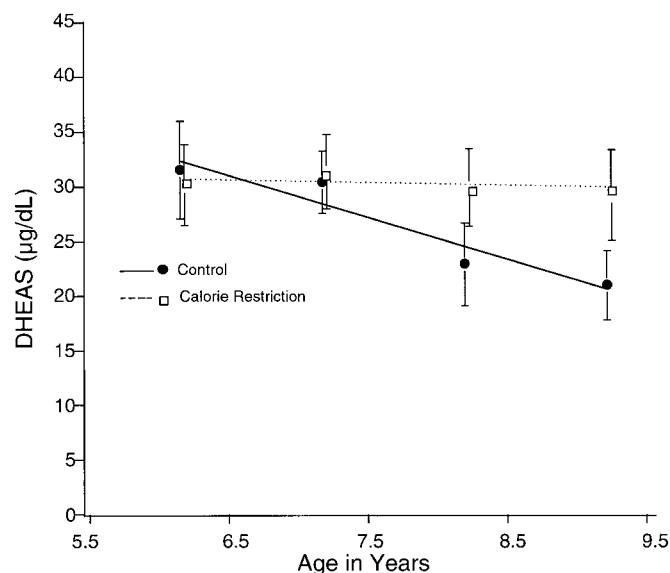


FIG. 7. Calorie restriction slows the age-related decline in serum dehydroepiandrosterone-sulfate (DHEAS) in adult male monkeys. The rate of change indicated by the slope of each regression line was significantly less for monkeys on calorie restriction, compared to controls ($t = 3.8$, $p < 0.005$). (Reprinted with permission from Lane, M. A. *et al.*, 1997, *J. Clin. Endo.* 3, 2093–2096).

mate studies of diabetes and cardiovascular disease risk have utilized obese controls or have altered the diet to contain a higher proportion of lipids. The finding that CR has potentially beneficial effects in lean, rather than obese, monkeys suggests that CR may have beneficial effects even in normal-weight individuals. In conclusion, although life span data are not yet available, it is likely that rhesus monkeys on CR will experience a moderate increase in life span and health span, due to the amelioration of complications associated with certain age-associated diseases.

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