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Physiology

Calorie restriction lowers body temperature in rhesus monkeys, consistent with a postulated anti-aging mechanism in rodents

(aging/*Macaca mulatta*/locomotor activity/circadian rhythm/heart rate)

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ABSTRACT **Many studies of caloric restriction (CR) in rodents and lower animals indicate that this nutritional manipulation retards aging processes, as evidenced by increased longevity, reduced pathology, and maintenance of physiological function in a more youthful state. The anti-aging effects of CR are believed to relate, at least in part, to changes in energy metabolism. We are attempting to determine whether similar effects occur in response to CR in nonhuman primates. Core (rectal) body temperature decreased progressively with age from 2 to 30 years in rhesus monkeys fed ad lib (controls) and is reduced by ≈0.5°C in age-matched monkeys subjected to 6 years of a 30% reduction in caloric intake. A short-term (1 month) 30% restriction of 2.5-year-old monkeys lowered subcutaneous body temperature by 1.0°C. Indirect calorimetry showed that 24-hr energy expenditure was reduced by approximately 24% during short-term CR. The temporal association between reduced body temperature and energy expenditure suggests that reductions in body temperature relate to the induction of an energy conservation mechanism during CR. These reductions in body temperature and energy expenditure are consistent with findings in rodent studies in which aging rate was retarded by CR, now strengthening the possibility that CR may exert beneficial effects in primates analogous to those observed in rodents.**

The only established intervention that significantly extends mean and maximal life-span in short-lived mammalian species is calorie restriction (CR), or undernutrition without malnutrition (1). In addition to CR's well-known effects on longevity, it also retards the onset of several age-related diseases and maintains most age-sensitive physiological functions at youthful levels (1–3). CR research is now focused on two questions: *(i)* What is the biological mechanism underlying the diverse and proven anti-aging effects of CR in rodents? and *(ii)* Will this intervention exert similar effects in longer-lived species, including humans? Despite extensive research, the possible biological mechanisms of CR remain unclear. To address the question of applicability of the CR paradigm to longer-lived species, in 1987 the National Institute on Aging began the first long-term study of the effects of CR imposed on rhesus and squirrel monkeys at several stages of their life-spans (4). Another study of adult-onset CR in rhesus monkeys was begun at the University of Wisconsin at Madison in 1989 (5).

Long-term CR in rhesus monkeys resulted in reduced body weight and altered body composition (4–9). Several other physiological changes, consistently found in rodents subjected to CR, also occur in restricted rhesus monkeys. For example, sexual (10) and skeletal (11) maturation are delayed by CR in rhesus monkeys. Beneficial effects of CR on glucoregulatory end points have also been documented in rhesus monkeys (8, 12). These initial studies of CR in nonhuman primates have shown that this experimental paradigm, so extensively used in rodents, can be safely applied in longer-lived species. Current findings from primate studies also demonstrate that certain biological outcomes related to possible mechanisms of action occur in both rodent and primate species subjected to CR.

Regardless of the mechanism by which CR retards the rate of aging, it is generally accepted that the effects of CR are attributed to a reduction in total energy intake (13). Since adult mammals use significant amounts of energy to maintain a relatively high, constant body temperature, it might be expected that reduced energy intake during CR could result in a lowered body temperature as the organism decreases metabolic rate and attempts to conserve energy. It is well-known that many small mammals respond to an energy deficit by reducing body temperature (14). Reduced body temperature has also been associated with severe undernutrition in humans (15). Several studies (1, 16) have documented a lowering of core body temperature in CR mice and, to a lesser extent, in rats (17, 18); it is possible that reduced body temperature may be involved in the mechanism of CR (16, 19). However, it has not been established whether such a reduction in body temperature occurs during long-term CR in longer-lived mammals such as nonhuman primates.

We report here the findings of two studies of rhesus monkeys on CR. In the first experiment, we examined the effects of long-term CR (6 years) on rectal temperature in monkeys of different ages. In a second experiment, we used radiotelemetry implants to assess circadian patterns of subcutaneous body temperature, locomotor activity, and heart rate during the gradual institution of short-term CR. Twenty-four hour energy expenditure was also measured using indirect calorimetry to quantitate oxygen consumption.

MATERIALS AND METHODS

Animal Care and Housing. The first experiment consisted of 27 male rhesus monkeys *(Macaca mulatta)* that began CR in April 1987. Three age groups representative of the species life-span were studied. Half of the monkeys in each group were fed approximately *ad lib* and the other half was placed on a 30% reduced feeding regimen as described (4, 11). Age groupings at the beginning of the study were as follows: group J [juveniles, ages 0.6–1.0 year), $n = 12$ (6 control and 6 CR)]; group A [adolescents, ages 3–5 years, $n = 12$ (6 control and 6

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Abbreviation: CR, calorie restriction.
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CR)]; and group O (old, ages 18–25 years, $n = 3$ control). Group O contained no monkeys on CR. The second, short-term study consisted of six young (ages 2.0 ± 0.1 years) male rhesus subjected to CR following baseline evaluation.

More detailed descriptions of animal history and housing can be found in previous articles (4, 6, 9). Briefly, 12 hr light/12 hr dark cycles (6 a.m.–6 p.m.), 22–28°C ambient temperature, and 50–60% relative humidity conditions were maintained since the onset of the study in 1987. Except for group O monkeys, which were housed individually, all other monkeys were housed as social pairs at the beginning of the study. Due to increasing frequency of aggressive encounters between cage mates, many animals had to be housed individually, such that by year 6 of the study all animals in the groups reported here were housed individually. Housing conditions in the second experiment were identical to those described above except that all monkeys were individually housed for the duration of the study.

Diet and Feeding Strategy. Diets for both studies were identical: a semi-synthetic formulation specifically designed to meet nutrient requirements for rhesus monkeys as recommended by the National Research Council (20). However, certain vitamins, minerals, and trace elements were included above the recommended levels (see refs. 4 and 11 for details). Thus, diet composition was not different between studies or between control and CR groups. The nutrient concentrations of the diet were 15.4% crude protein, 5% crude fat, 8% fiber, and 3.77 kcal/g energy.

To maintain more precise control of food intake, all monkeys were fed specified amounts of food in two meals (7:00 a.m. and 2:00 p.m.) each day. Daily food allotments for control monkeys were based on age and body weight and followed established National Research Council guidelines (20). Because control monkeys in the first study were given prescribed daily allotments and not permitted free or unlimited access to food, they were not fed *ad lib*. However, regular assessment of food consumption over the course of the study has shown the offered allotments to approximate ad lib consumption (E.M.T., unpublished data). Meal allotments for CR animals were 30% less than that of control monkeys of comparable ages and body weights.

In both studies, CR was gradually instituted over a period of 3 months. In the first study, the level of restriction was 10% during the first 4-week interval, 20% during the second 4-week interval, and 30% for the third 4-week interval and thereafter. In the second study, monkeys were randomly assigned to one of two measurement groups at the start of the experiment. Group 1 (animals 346, 446, and 463) began CR 30 days before group 2 (animals 548, 747, and J226). *Ad lib* food consumption was determined for each monkey before CR initiation. After 30 days of *ad lib* consumption, food allotments were reduced 10% and maintained at that level for 30 days. Additional reductions to 20 and 30% CR were conducted in a similar manner.

Experimental Measurements. In the first study, measurements of rectal body temperature (t_R) were obtained quarterly. After an overnight fast, monkeys were anesthetized with ketamine (7–10 mg/kg i.m.); t_R was measured between 8:00 a.m. and 10:00 a.m. and within 10 min after ketamine administration. A rectal probe was inserted 4–6 cm and the temperature was recorded as t_R.

Before the start of the second experiment, a Datasciences (Minneapolis) model TA10CATA-D70-L60 radiotelemetry transmitter, calibrated according to the manufacturer's specifications, was surgically implanted under the skin of each anesthetized monkey near the lower lateral margin of the rib cage. After implantation surgery, the monkeys were allowed to recover for 1 month before the *ad lib* phase of the experiment. All physiological measurements were obtained after monkeys had been fed at each level of intake for 30 days. During each measurement period, subcutaneous body temperature (t_{sc}), heart rate, and locomotor activity were continuously recorded for 7 days. All telemetry measurements were recorded using the DATAQUEST IV data acquisition program (Datasciences) while monkeys were in their home cages under normal living conditions.

On separate days, 24-hr energy expenditure was measured by indirect calorimetry using an open-circuit, flow-through system. A standard rhesus cage (88.9 × 61.0 × 68.5 cm) was placed in an airtight chamber. Each monkey was allowed to adapt to this environment for 48 hr before measurement. Air flow through the chamber was measured by using a volume displacement flowmeter. Chamber temperature was maintained by using a thermostatically controlled cooling unit. Incoming and expired gases were collected in Tedlar bags. CO_2 and O_2 concentrations were measured by using infrared and zirconia cell analyzers, respectively. Energy expenditure was calculated by the Weir equation (21).

Estimates of lean body mass were obtained for monkeys in the second study by using dual-energy x-ray absorptiometry (Lunar DPX-α, Lunar, Madison, WI). Details of this technique, which assesses various tissue components from the relative attenuation of two energies of x-rays, have been described (22). For dual energy x-ray absorptimetry scans, monkeys were deprived of food overnight and anesthetized with Telazol (3.5 mg/kg i.m.). Total body scans (mode = P 4 − 12, elapsed time ~10 min) were done with the monkey in a supine position on the scan table. Lunar PEDIATRIC SOFTWARE (version 1.3e) was used for all scanning and data analyses.

Statistical Analyses. In the first study, the effects of age and CR on body temperature were assessed by using a three (age) by two (diet) ANOVA with repeated measures. For the second study, effects of intake amounts (0–30% CR) on the data acquired by telemetry were evaluated by using one-way ANOVA with repeated measures. Finally, CR-induced changes in energy expenditure were assessed by calculating the linear regression of energy expenditure onto the number of months on CR and energy expenditure. For all analyses, statistical significance was accepted as $P < 0.05$.

RESULTS

Effects of Long-Term CR on t_R. Reduced t_R in monkeys on long-term CR was evident after the second year of the study. Longitudinal measurements of t_R during long-term CR (experiment 1), summarized as annual values, are presented in Fig. 1. It is apparent that t_R, measured under ketamine anesthesia after an overnight fast, was lower in older monkeys (group O) as confirmed by a significant effect of age on t_R [$F(2,25) = 21.2$; $P < 0.0001$]. Beginning in the second year of the study, t_R decreased in CR monkeys such that mean t_R was approximately 0.5°C lower than control monkeys. This observation was confirmed by a significant effect of CR on t_R over the 6-year study [$F(1,20) = 5.06$; $P < 0.03$].

Effects of Short-Term CR on 24-hr t_{sc}. When 24-hr temperature was monitored with telemetered probes, t_{sc} decreased significantly after caloric intake was reduced by 30% of *ad lib* amounts. Results of the second experiment are presented in Figs. 2 and 3. No statistical differences were observed between measurement groups 1 and 2 for any measured variables; therefore, the groups were combined for further analysis. Fig. 2 summarizes circadian patterns of t_{sc} during *ad lib* feeding and for 2 consecutive months CR at 30%. t_{sc} tended to be lower at 10% and 20% CR (data not shown) compared with *ad lib*, but significant differences did not emerge until monkeys were restricted by 30%. Temperatures peaked at midday and were lowest at night. In addition, 30% CR significantly reduced mean t_{sc} in this group. This was confirmed by a significant effect of CR on 24-hr measurements of t_{sc} [ANOVA repeated measures, $F(3,20) = 6.5$; $P < 0.003$]. Room temperature monitors confirmed that ambient temperature was not significantly altered during the entire study (M.A.L., unpublished data). Fig. 3 summarizes the response of t_{sc} in individual monkeys when begun on 30% CR. All monkeys

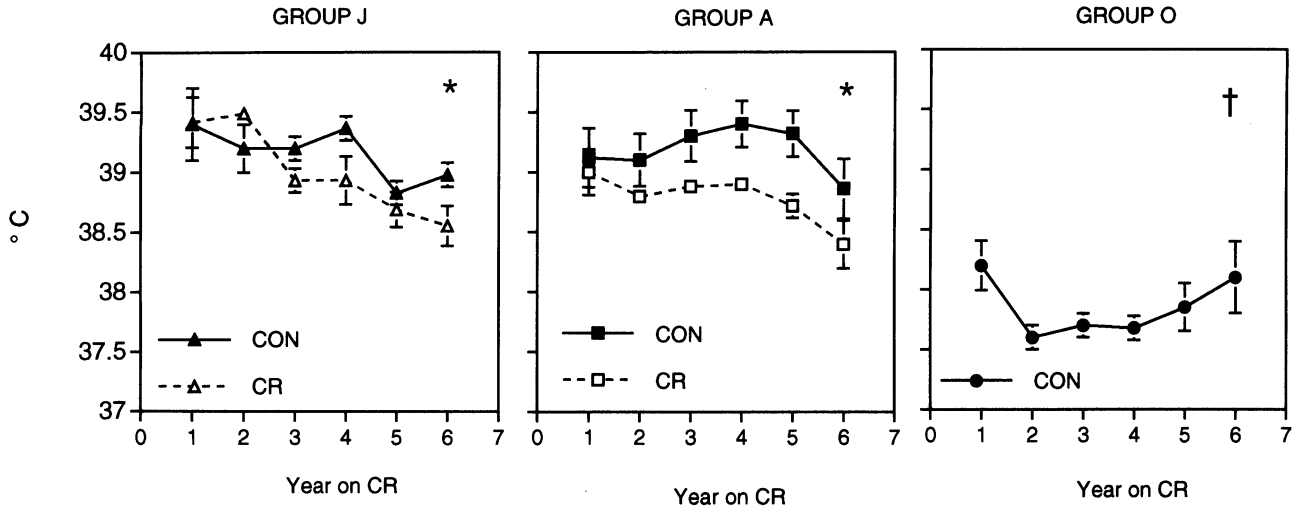

FIG. 1. Effect of age and CR on colonic body temperature (t_R) in control (CON) and CR (CR) monkeys in each of three age groups (J, A, and O). Each point represents the mean yearly value (±SEM) of t_R for monkeys. The main effects of age *(Right)*, $F(2,25) = 21.2$, $P < 0.0001$ and CR *(Left and Center)* $F(1,20) = 5.06$, $P < 0.03$ were significant (ANOVA repeated measures).

responded to 30% CR in a qualitatively similar manner. Although differences in magnitude exist, t_{sc} was lowered in all monkeys once they reached 30% CR (P values < 0.05).

Heart rate and locomotor activity data are summarized in Fig. 4. The circadian patterns for heart rate and locomotor activity exhibited the expected diurnal patterns during the overnight hours, but there was no significant effect of CR on overall activity. However, it should be noted that monkeys that had been on CR for at least 1 month were noticeably more active in the hours preceding meal time (Fig. 4). Similar effects on locomotor activity have been reported for rhesus monkeys in our long-term study (7).

Energy Expenditure. Gradual reduction of food intake (10% per month) caused a progressive decline in 24-hr energy expenditure as measured by indirect calorimetry. Fig. 5 shows that absolute energy expenditure and energy expenditure per lean body mass were progressively lowered at each intake amount compared with *ad lib* feeding. By the fourth month of the CR regimen, estimates of absolute and relative (per kg of lean mass) energy expenditure were reduced (P values < 0.05) by about 24% from baseline levels.

DISCUSSION

These studies show that a dietary regimen that is known to increase life-span in rodents and other short-lived species lowered body temperature in a long-lived nonhuman primate. Results of the long-term longitudinal study demonstrate that this reduction in temperature is maintained over several years. Results of the short-term experiment show that reductions in body temperature occur after CR is imposed in young rhesus monkeys. Concomitant reductions in 24-hr energy expenditure, but not locomotor activity or heart rate, were observed.

Our finding that body temperature was reduced in rhesus monkeys subjected to long-term CR agrees with several reports of similar findings in rodents (16–18, 23–25). Some studies have failed to document a CR-induced reduction in rodent body temperature (26–28). However, in these studies temperature was measured by using a rectal probe and some form of restraint, either by manual restraint to obtain the measurement or in a special apparatus during a cold-stress procedure. The additional physiological stress induced as a result of these procedures could have contributed to the negative findings.

The magnitude of difference between body temperatures of control and CR rhesus monkeys on long-term CR was somewhat less than that reported in most mouse studies. Furthermore, differences in t_R were not apparent until after the second year of CR. Possible explanations for these differences include time of measurement relative to meal time, use of a single time-point measurement, and stress induced during the procedure. Specifically, we recorded a single measurement of body temperature during the morning hours at approximately the same time animals would have been fed their morning meal. It is possible that using different, and more frequent, measurement times (as in the short-term experiment) could have revealed more marked differences between control and CR animals earlier in the study. It is also possible that the use of ketamine anesthesia influenced the measurements. Previous rodent studies (18, 25) and the findings of our short-term study suggest that the temperature difference between control

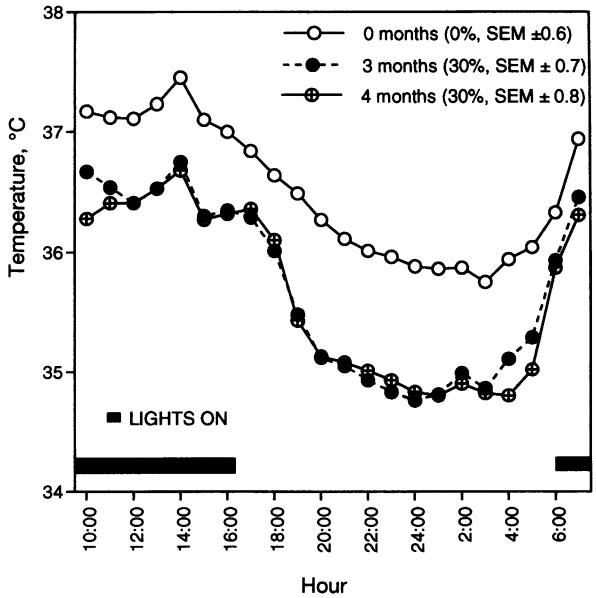

FIG. 2. Circadian patterns of subcutaneous body temperature (t_{sc}) during institution of CR in young (2 yr) rhesus monkeys. Each point represents the mean hourly temperature (t_{sc}) measured continuously for 7 days for all monkeys ($n = 6$) at baseline (0 months) and at 3 and 4 months after initiation of CR. Numbers in parentheses represent the percent restriction and SEM. There was a significant main effect of intake amount on 24-hr measurements of t_{sc} (ANOVA repeated measures) [$F(3,20) = 6.5$, $P < 0.003$].

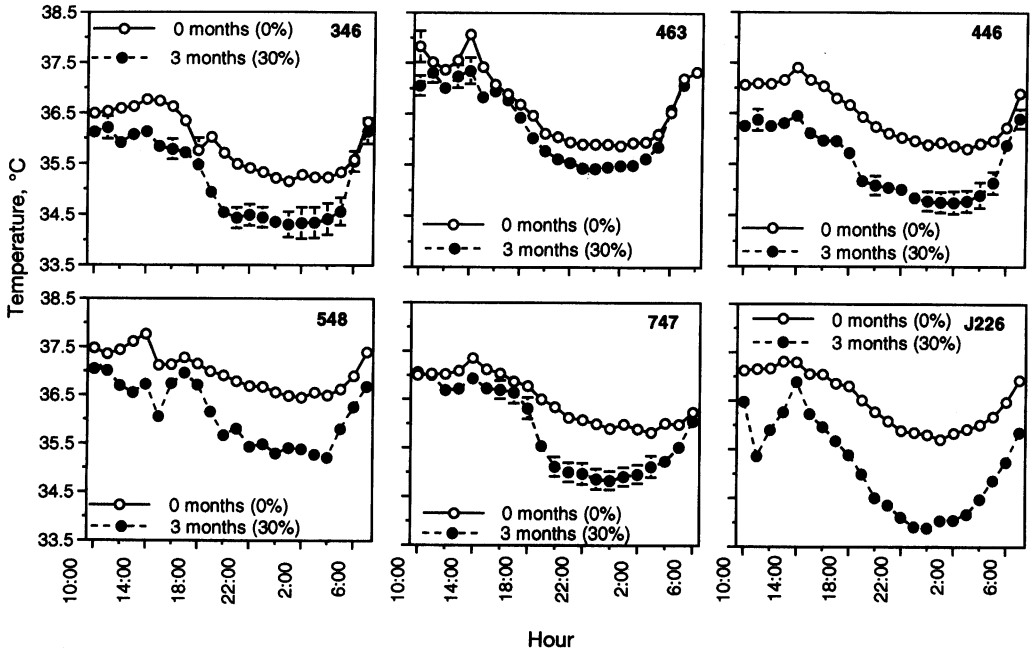

FIG. 3. Subcutaneous body temperature (t_{sc}) during ad lib (baseline, 0 months) feeding (0%) and CR (3 months, 30% CR) for all monkeys in the second study. Each point represents the mean (±SEM) hourly t_{sc} measured continuously for 7 days. Numbers in parentheses represent the percentage restriction of calorie intake. Error bars not shown are too small for graph scale.

and CR animals is greatest in the hours just before the regularly scheduled feeding time. Therefore, the use of a single, mid-morning (after normal meal time), measurement of t_R in ketamine-anesthetized monkeys could have influenced our findings in this longitudinal study.

Results of the short-term experiment show that gradual reduction of intake to 70% of *ad lib* amounts resulted in significantly reduced t_{sc} during the entire day as compared with t_{sc} measured during *ad lib* feeding. To our knowledge, similar studies in rodents using an identical feeding and measurement strategies have not been reported. However, our findings agree with the majority of rodent CR studies (long- and short-term) for which body temperature data were reported. Direct comparison with human data is not appropriate because the degree of restriction and the experimental design differed significantly. However, it should be noted that our findings are in general agreement with data reported from undernutrition studies in humans (15). These findings suggest that adaptation to CR in rhesus monkeys is similar to that reported in rodents. It is unlikely that period or cohort effects contributed to our findings, as monkeys in each measurement group exhibited similar responses to CR. Whether similar changes in the diurnal pattern of body temperature occur during long-term CR must await future study.

There is considerable evidence supporting the idea that an initial response to reduced intake is decreased energy expenditure that occurs before any adjustments in metabolic mass are seen. In fact, Garrow (29) has stated "there is no investigator who has looked for this effect and failed to find it." Therefore, our finding that 24-hr energy expenditure was reduced during short-term CR was not unexpected. These findings are consistent with several rodent studies that reported metabolic rate was reduced immediately following the initiation of restricted feeding begun just after weaning (17, 30, 31). In these studies, energy expenditure was reduced immediately after initiation of reduced intake, but it remained lowered only for about 6 weeks. McCarter (32) suggests that different results would be expected if CR were initiated later in life and energy expenditure would remain lower in CR animals for an extended period. Indeed, studies of adult-onset CR in rodents (33, 34) and primates (35) support this suggestion. It is not clear whether the latter findings are at odds with the work of McCarter and colleagues (30, 31) or simply

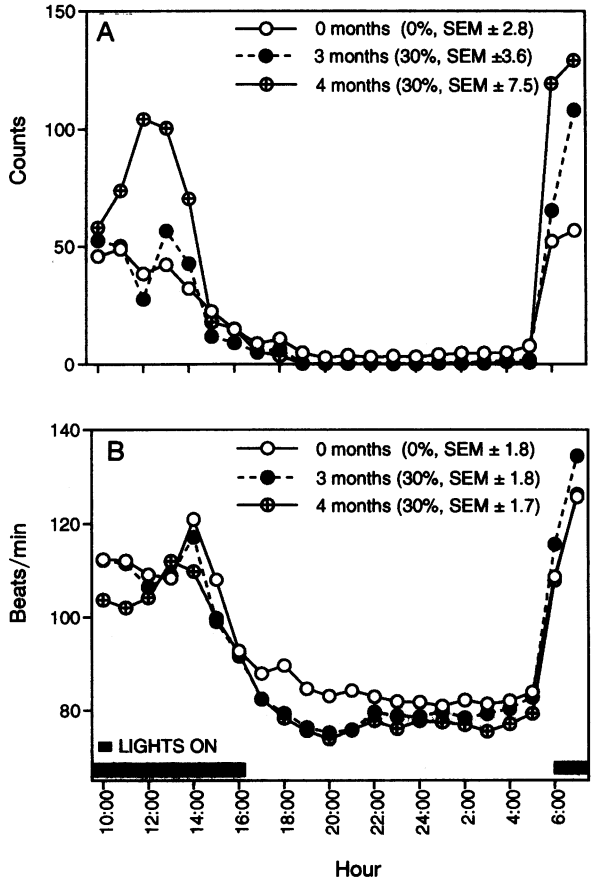

FIG. 4. Circadian patterns of locomotor activity *(A)* and heart rate *(B)* measured by radiotelemetry during institution of CR. Each point represents the mean hourly activity or heart rate for all monkeys (n = 6) at a given intake level. Numbers in parentheses represent the percent restriction and SEM.

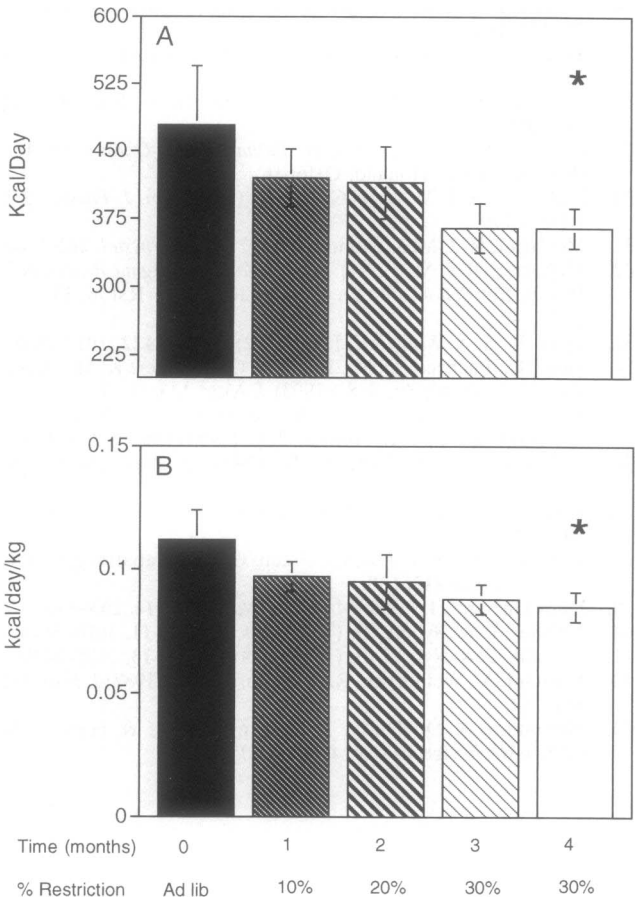

FIG. 5. Effect of CR on absolute energy expenditure *(A)* and energy expenditure per kg of lean body mass *(B)*. Each bar represents the mean (±SEM) energy expenditure at each feeding level as determined by indirect calorimetry. Linear regressions for absolute energy and energy expended per kg of lean mass were significant (P values < 0.05).

represent differences in experimental design or animal body composition. The present findings suggest that young monkeys on short-term CR rapidly reduced energy expenditure to compensate for the reduction in energy intake. Reductions in energy expenditure might be due to decreased body temperature, locomotor activity, or changes in organ size, resulting in reduced basal metabolism. In this study, it was not possible to measure organ size or basal metabolic rate. However, since heart rate and locomotor activity were not altered by CR, it is likely that most of the reduction in energy expenditure relates to decreased body temperature. Our findings suggest that reduced energy expenditure related to a reduction in body temperature represents a major energy-sparing mechanism following the initiation of CR.

Reductions in body temperature could relate to potential metabolic mechanisms of CR-induced increases in longevity. For example, several studies in poikilothermic invertebrates and vertebrates have demonstrated that life-span can be extended by reducing ambient temperature (1). Similar studies in homeotherms have been limited to rats and do not report increases in life-span associated with exposure to decreased environmental temperatures (36, 37). However, reduced body temperature during restricted energy intake has been reported in several rodent species. Studies in hibernating mammals, which enter a period of torpor (reduced body temperature), also suggest a link between temperature and life-span. Lyman *et al.* (38) demonstrated that Turkish hamsters that spent 11–33% of their lives in hibernation lived 23% longer than hamsters that never hibernated. The European viper *(Viper aspis)*, which usually lives 15–18 years if permitted to enter hibernation, normally will live no longer than 10 years if hibernation is prevented (39).

The exact relationship between reduced body temperature and increased longevity as seen in CR and hibernation studies is not known. However, it is interesting to note that certain molecular events, which could influence longevity, biological aging processes, or age-related diseases, are altered by CR and correlated with body temperature. For example, reduced body temperature in CR animals would likely lead to decreased DNA damage (40–42). Other studies have shown that expression of protooncogenes (c-*myc*) associated with certain kinds of neoplasia was reduced in CR mice. Furthermore, c-*myc* expression and body temperature are at their lowest daily value during the same circadian stage (43).

None of these findings establish a clear link between metabolic consequences of CR, energy conservation, and reduced body temperature. However, these studies suggest the need for further investigation of metabolic processes during CR. It is apparent from our findings that rhesus monkeys on CR exhibit energy conservation mechanisms that are very similar to those reported in rodents. Our findings suggest that changes in body temperature, as they relate to metabolism in general, might represent a primary mechanism responsible for the anti-aging effects of CR. If such shifts in energy metabolism are found to relate to basic metabolic mechanisms of CR, then we can expect that longer-lived species, such as nonhuman primates, subjected to CR would exhibit many of the anti-aging anti-disease effects seen in rodents.

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