Energy Restriction Does Not Alter Bone Mineral Metabolism or Reproductive Cycling and Hormones in Female Rhesus Monkeys

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ABSTRACT Energy restriction (ER) extends the life span and slows aging and age-related diseases in short-lived mammalian species. Although a wide variety of physiological systems have been studied using this paradigm, little is known regarding the effects of ER on skeletal health and reproductive aging. Studies in rhesus monkeys have reported that ER delays sexual and skeletal maturation in young male monkeys and reduces bone mass in adult males. No studies have examined the chronic effects on bone health and reproductive aging in female rhesus monkeys. The present cross-sectional study examined the effects of chronic (6 y) ER on skeletal and reproductive indices in 40 premenopausal and perimenopausal (7–27 y old) female rhesus macaques (*Macaca mulatta*). Although ER monkeys weighed less and had lower fat mass, ER did not alter bone mineral density, bone mineral content, osteocalcin, 25-hydroxyvitamin D, 1,25-hydroxyvitamin D or parathyroid hormone concentrations, menstrual cycling or reproductive hormone concentrations. Body weight and lean mass were significantly related to bone mineral density and bone mineral content at all skeletal sites (total body, lumbar spine, mid and distal radius; $P \leq 0.04$). The number of total menstrual cycles over 2 y, as well as the percentage of normal-length cycles (24–31 d), was lower in older than in younger monkeys ($P \leq 0.05$). Older monkeys also had lower estradiol ($P = 0.02$) and higher follicle-stimulating hormone ($P = 0.02$) concentrations than did younger monkeys. We conclude that ER does not negatively affect these indices of skeletal or reproductive health and does not alter age-associated changes in the same variables. J. Nutr. 131: 820–827, 2001.

KEY WORDS: • *aging* • *bone loss* • *primate* • *reproduction* • *energy restriction*

Energy restriction (ER), or the chronic restricted intake of a nutritionally replete diet, remains the only means of reproducibly extending the life span and retarding physiological aging and age-related disease (1,2). This paradigm has been studied most extensively in mice, rats and other short-lived species: however, investigations in nonhuman primates (3,4) are beginning to yield promising data demonstrating that physiological responses of rhesus monkeys to ER closely parallel existing rodent literature (5) and that ER reduces many risk factors associated with diabetes and cardiovascular disease (6,7) and may even reduce mortality rates related to these diseases (8).

With few exceptions, ER is thought to retard many of the physiological changes that occur with aging. Bone loss and reproductive aging are among the most important public health issues for older individuals, but little information is available regarding the possible effects of ER on these aspects of aging. The initial reports that this regimen extended the life span in rodents also recognized the potential for a negative influence on bone mass (9). In this study, it was noted that femurs removed from severely ER rats were extremely fragile and reportedly crumbled on dissection. Subsequent studies have used less severe levels of ER (60% of ad libitum, or 40% ER) and have reported reductions in several indices of skeletal mass such as bone mineral density, bone mineral content, calcium content and bone strength (10–13). Only two studies have examined the effect of ER (40%) on bone loss with aging in rats (14,15), and they reported that unlike their counterparts who were fed ad libitum, ER rats did not experience significant reductions in bone mass between 24 and 27 mo of age.

Data regarding the effects of chronic ER on reproductive indices are similarly limited. ER, such that the body weight of restricted rats remained at 50% of that of controls, delayed the onset of puberty (16,17) and retarded the loss of ovarian function (acyclicity) that occurs during aging (16,18). The onset of persistent estrus was delayed in ER (40%) female rats (19). ER (20–40%) mice become acyclic (20,21), but on refeeding, they continue to cycle at ages beyond which control

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[2] Abbreviations used: BMC, bone mineral content; BMD, bone mineral density; CON, control; DPD, deoxypyridinoline; DEXA, dual energy x-ray absorptiometry; ER, energy restricted (restriction); FSH, follicle-stimulating hormone; LH, leutinizing hormone; 25(OH)-D, hydroxyvitamin D; 1,25-$(OH)_2$-D, 1,25-dihydroxyvitamin D; PYD, pyridinoline; RIA, radioimmunoassay.

Manuscript received 16 June 2000. Initial review completed 21 August 2000. Revision accepted 12 December 2000.

mice that are fed ad libitum had ceased normal estrous cycling. Although limited, studies of reproductive hormones during ER demonstrate that in young female rats, ER reduced serum follicle-stimulating hormone (FSH) and progesterone concentrations while increasing 17β-estradiol concentrations measured during the first estrous cycle (17). In older rats (220 d), ER reduced the estradiol peak and levels of progesterone, but FSH concentrations reportedly increased (18). Sampling in these studies was synchronized to the phase of estrous, which was determined through vaginal smears.

Several studies have demonstrated the possible use of nonhuman primate models for studies of skeletal and reproductive aging. For example, nonhuman primate models of both estrogen depletion (22–29) and age-related bone loss (30–37) have been described. In addition, many nonhuman primate species, rhesus monkeys in particular (38,39), exhibit a natural menopause that includes oligomenorrhea followed by a complete cessation of menstrual cycling and changes in reproductive hormones that parallel findings in humans. However, studies of the effect of ER, which retards many aging processes, on skeletal and reproductive indices in longer-lived species are limited to male rhesus monkeys. We reported that ER delayed both sexual (40) and skeletal (41) maturation in young male rhesus monkeys. A more recent study (42) from our laboratory reported that male rhesus monkeys on longer-term ER (>11 y) exhibited a slight reduction in bone mass at some (mid and distal radius), but not all (total body and lumbar spine), skeletal sites and that the effects of ER were mostly due to changes in body composition. A study of adult-onset ER at the University of Wisconsin (4) reported that after 12 mo of ER, there was no significant reduction in bone mass in male monkeys compared with control (CON) monkeys. No studies have reported the effects of ER on skeletal health and reproductive function in female rhesus monkeys.

The present study was designed to assess skeletal mass, reproductive cycling and related biochemical markers in young, adult and perimenopausal female rhesus monkeys after 6 y of ER. We used dual-energy x-ray absorptiometry (DEXA) to assess bone mineral density (BMD) and bone mineral content (BMC) at several skeletal sites, including total body, lumbar spine and the mid and distal radius. Several indices of skeletal metabolism and reproductive function obtained from serum samples were also studied, including bone turnover, calcium homeostasis and reproductive cycling and hormones.

MATERIALS AND METHODS

Animals. A total of 40 female rhesus monkeys (*Macaca mulatta)* ranging in age from 7 to 27 y were used in this study. All monkeys consumed the same semisynthetic, pelletized feed (NIA open formula; Ralston Purina) offered in two meals each day at 0700 and 1400 h. Daily food allotments for CON monkeys followed established primate nutrition guidelines (43) for monkeys of a given age and weight. ER monkeys were offered 30% less food per day than CON monkeys of a comparable age and body weight. ER was begun 6 y before the collection of data for the present study. At the time ER was initiated, monkeys ranged in age from 1 to 21 y. Regular measurements of food consumption during the study have shown that the specified allotments offered to CON monkeys approximated the amount eaten by providing free access to food.

To ensure adequate micronutrient intake in the monkeys eating 30% less food per day, the diet was formulated to exceed National Research Council guidelines (43) for vitamins, minerals and trace elements by ~30–40%. Therefore, because the diet composition was the same for the CON and ER groups, the experimental manipulation was a reduction in total intake (including all nutrients) and not an alteration in or a deficiency of a specific dietary component. The nutrient content of the diet was 173 g protein/kg, 50 g fat/kg, 65 g fiber/kg and 16.32 kJ/g gross energy. Calcium, phosphorus and vitamin D concentrations of the diet were 12.2 g/kg, 6.1 g/kg and 3.5 IU/g, respectively. Because the daily food allotments were greater in CON monkeys, the absolute intakes of calcium, phosphorus, vitamin D and all other nutrients were higher ($P < 0.0001$) in CON than in ER monkeys (1.8 ± 0.07 versus 1.3 ± 0.04 g/d, 0.19 ± 0.04 versus 0.08 ± 0.02 g/d and 491.6 ± 20.2 versus 363.8 ± 9.8 IU/d, respectively). Additional details on diet composition and food allotments and consumption can be found in previous reports (3,41).

Female rhesus monkeys reach puberty at 2.5–3.5 y of age, have a mean life span of 24 y (44) and experience reproductive changes similar to human menopause in their mid-20s (39). Thus, monkeys in the present study began ER at various life stages, including prepubertal, adult and old adult. At the onset of this study, the monkeys were matched for age and body weight and divided into two groups: CON ($n = 21$) and ER ($n = 19$). Age ranges at which data where collected in the present study and means for the two groups are presented in **Table 1**. The monkeys included in this study were acquired in 1991 from two sources (a military testing program at Aberdeen Proving Ground, Maryland, and Labs of Virginia, Yemassee, SC). Monkeys were born in captivity and thus had known birth dates and well-documented histories. No monkey had been involved in invasive experimentation before entering the study. These animals are part of an ongoing longitudinal investigation of ER and aging at the National Institute on Aging (NIA) (3,45). No monkey in the present study had been acyclic for >6 mo, and therefore all were considered to be premenopausal during the period of data collection.

Housing. Housing and feeding conditions were identical to those described in our longitudinal study of aging in male rhesus monkeys (3,45). Briefly, monkey rooms were maintained on a 12-h light/dark cycle (lights on 0600–1800 h) with room temperature of 22–28°C and 50–60% humidity. All animals have been housed at the Primate Unit of the National Institutes of Health Animal Center since the beginning of the study. The Primate Unit is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and all procedures described herein received full approval of the Animal Care and Use Committee of the Gerontology Research Center, National Institute on Aging, National Institutes of Health.

Data collection and laboratory determinations. All procedures were conducted in food-deprived (overnight), anesthetized with tilotamine HCl/zolazepam HCl (Telazol 3.5–4.0 mg/kg intramus-

TABLE 1

Subject characteristics and body composition in control (CON) and 30% energy-restricted (ER) female rhesus monkeys[1]

	n	Age range	Age	Energy intake	Body	Lean mass	Fat mass
		y		*kJ/d*	*kg*		
CON	21	7.4–25.4	13.4 ± 5.3	2346 ± 88.3	6.8 ± 1.8	5.2 ± 0.9	1.3 ± 1.0
ER	19	8.4–27.4	14.1 ± 6.8	1709 ± 51.5*	5.7 ± 1.2*	4.7 ± 0.6	0.7 ± 0.7*

[1] Values for age, lean mass and fat mass are means ± SEM.
* Significantly different from CON, $P \leq 0.05$.

cular) monkeys. If additional chemical restraint was required for DEXA scans, an inhalable anesthetic (isofluorane) was used and titrated to reduce all extraneous skeletal muscle movement. Total body, lumbar spine (lumbar vertebrae 2–4) and forearm (mid and distal radius) bone mineral density and content were determined by DEXA scans after 6 y of ER. All DEXA scans were performed with a Lunar DPX-α scanner (Lunar, Madison, WI) using Lunar Pediatric Software (Version 1.3 E) for total body and spine scans and Lunar Small Animal Software (Version 1.0C) for forearm scans. For scans of the radius, a forearm positioning board supplied by the manufacturer was used (Lunar, Madison, WI) to place the monkey's left arm (palm down, open fist) on the scan table. The scanned image of the radius was divided into three equal portions, and bone mass in the middle and distal thirds were determined. Precision values for the five DEXA sites are summarized in **Table 2**.

Blood samples for all biochemical variables except reproductive hormones were collected between 0800 and 1100 h, just before the DEXA scans, via single venipuncture of the femoral vein. Early follicular blood samples for reproductive hormones were collected during a 3-mo period around the DEXA scan.

Monkeys were observed daily for signs of menstrual bleeding. The first observation of bleeding was counted as d 1 of the cycle, and blood samples were then collected in anesthetized monkeys on d 5. Serum was separated by centrifugation and stored at −80°C for future analyses of biochemical parameters. Menstrual cycling was observed daily in two 12-mo periods before and after DEXA scans and blood sampling. The rhesus menstrual cycling is normally 24–31 d long (38).

Frozen serum samples were analyzed for osteocalcin [RIA (radioimmunoassay); Diagnostic Systems Laboratories, Webster, TX); parathyroid hormone (PTH) (IRMA; Diagnostic Products Corporation, Los Angeles, CA);1,25-dihydroxyvitamin D [1,25$(OH)_2$-D] (double antibody RIA; DiaSorin, Stillwater, MN) and 25-hydroxyvitamin D [25(OH)D] (RIA; DiaSorin). Estradiol, progesterone, FSH and luteinizing hormone (LH) concentrations were analyzed at Wisconsin Regional Primate Research Center (Madison, WI) with in-house RIA. Serum concentrations of calcium (colorimetric assay, Hitachi 747) and phosphorus (kinetic) were measured at Antech Diagnostics (Farmingdale, NY).

Mid-morning voided urinary samples were collected and assayed for pyridinoline (PYD) and deoxypyridinoline (DPD), markers of bone resorption. Both were measured with HPLC. Peaks were detected by fluorescence, quantified by external standards (courtesy of S. Robins Rowett Research Institute, Aberdeen, Scotland) and expressed per mmol of urinary creatinine (No. 555; Sigma Chemical Co., St. Louis, MO).

Statistical analyses. Cross-sectional effects of aging on BMC and BMD in CON monkeys were assessed through simple linear regression. A Student's nonpaired *t* test was initially used to determine the effects of ER on the same measures. ANCOVA was used to assess the independent effects of ER, age and body weight on bone mass. For all data, normality of residuals was tested. Most data met normality assumptions. In those isolated cases when data did not satisfy these assumptions, data were transformed using a Box-Cox procedure (46). In the cases where data were transformed to approximate normality, a linear transformation was subsequently applied to return the data to original scale to simplify interpretation. All analyses were conducted with NCSS Statistical Software (Kaysville, UT) with $P \leq 0.05$ accepted as significant. Homogeneity of regression (parallelism of slopes) was tested across the CON and ER conditions to evaluate the validity of using ANCOVA-based inferences regarding ER effects. Identical techniques were used to explore relationships between bone mass (dependent variables BMD and BMC) and measures of body composition (independent variables lean mass and fat mass) as well as the effects of age and ER on various biochemical variables, hormones and menstrual cycling.

TABLE 2

Dual-energy x-ray absorptiometry scan precision in rhesus monkeys[1]

	BMD[2]	BMC
	%	
Total body	1.06	0.91
Lumbar spine (L2–4)	1.00	1.27
Mid radius	1.45	1.63
Distal radius	1.79	1.39

[1] Precision, indicates mean coefficient of variation (%) of five scans repeated with repositioning on five rhesus monkeys.
[2] BMC, bone mineral content; BMD, bone mineral density.

TABLE 3

Effects of 30% energy restriction (ER) on bone mass in control (CON) and ER female rhesus monkeys[1]

Site	CON	ER	*P*-value[2]
Bone mineral density, *g/cm²*			
Total body	0.76 ± 0.01	0.75 ± 0.01	0.53
Lumbar spine (L2–4)	0.72 ± 0.02	0.69 ± 0.02	0.28
Mid radius	0.42 ± 0.01	0.41 ± 0.01	0.58
Distal radius	0.37 ± 0.01	0.36 ± 0.01	0.63
Bone mineral content, *g*			
Total body	253.86 ± 10.02	234.42 ± 9.66	0.19
Lumbar spine (L2–4)	7.65 ± 0.28	7.12 ± 0.39	0.27
Mid radius	1.09 ± 0.05	1.01 ± 0.04	0.16
Distal radius	1.01 ± 0.04	0.95 ± 0.03	0.29

[1] Values are means ± SEM for CON ($n = 21$) and ER ($n = 19$) female monkeys.
[2] *P*-values indicate results of Student's *t* test analysis between CON and ER monkeys.

RESULTS

Table 1 summarizes various subject characteristics, including age ranges for CON and ER groups, and energy intake and the effects of ER on body weight and composition. Group size and the age range studied did not differ between CON and ER groups. As expected, female monkeys in the ER group consumed fewer calories, weighed less and had less fat mass than CON monkeys ($P < 0.05$).

BMC and BMD tended to be lower in ER monkeys than in CON monkeys (**Table 3**). Although linear regression did not detect any cross-sectional age effects on BMC or BMD (**Figs. 1, 2**), when ANCOVA was used to assess the independent effects of ER, age and body weight on bone mass, a significant independent effect of age on BMD, but not BMC, was observed at all skeletal sites (**Table 4**; $P < 0.05$). There also was an independent effect of body weight on all measures of bone mass. These data confirm those in Table 3 in that, with one exception (total body BMD, $P < 0.05$), there were no independent effects of ER on bone mass with control for age and weight.

The relationship among bone mass, diet and body composition (fat and lean mass) was also examined (**Table 5**). Lean body mass was a significant predictor of bone mass at all sites ($P < 0.05$). Fat mass was correlated only with mid and distal radius BMC ($P \leq 0.03$, respectively).

Data on various biochemical markers relevant to skeletal metabolism are presented in **Table 6**. Serum concentrations of parathyroid hormone and 1,25$(OH)_2$-D were unaltered by age.

Serum 25(OH)-D concentrations were lower in older female monkeys ($P < 0.05$). No effects of ER were observed for any biochemical variable.

Figure 3 summarizes the effects of age and ER on serum estradiol, FSH, progesterone and LH. Only estradiol and FSH showed significant cross-sectional changes with age, with lower estradiol and higher FSH concentrations in older monkeys relative to younger monkeys. No significant effects of chronic ER on reproductive hormone concentrations were observed.

The total number of menstrual cycles (**Fig. 4***A*) and the percentage of normal length (24–31 d) cycles (Fig. 4*B*) showed a significant cross-sectional decline with age in CON monkeys ($P < 0.05$). There were no effects of ER on either of these cycling variables.

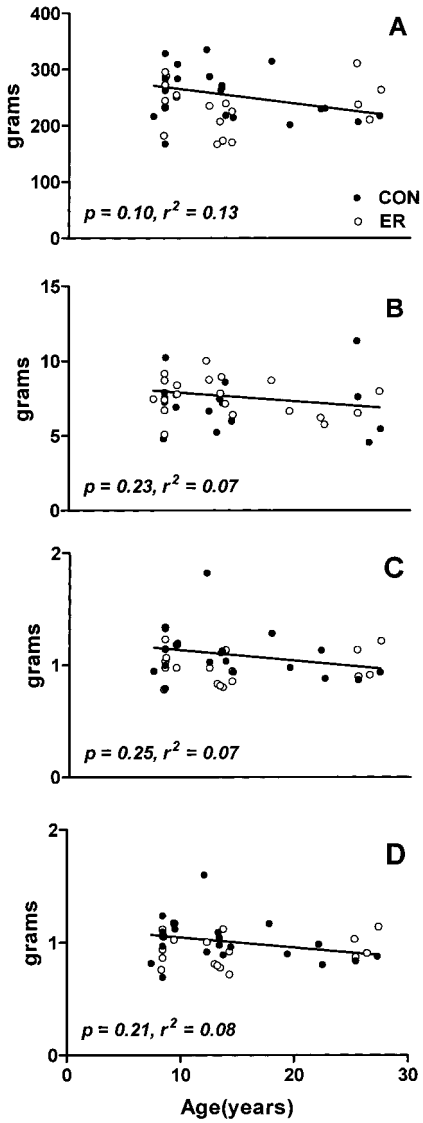

FIGURE 1 Total body (*A*), lumbar spine (*B*) mid radius (*C*) and distal radius (*D*) bone mineral content (BMC) in control (CON, $n = 21$) and 30% energy restricted (ER, $n = 19$) female rhesus monkeys. Each point represents BMC for individual monkeys at the corresponding age. Linear regression analysis revealed no significant cross-sectional effects of age on BMC. ER did not affect BMC at any site. Data were collected after 6 y of ER.

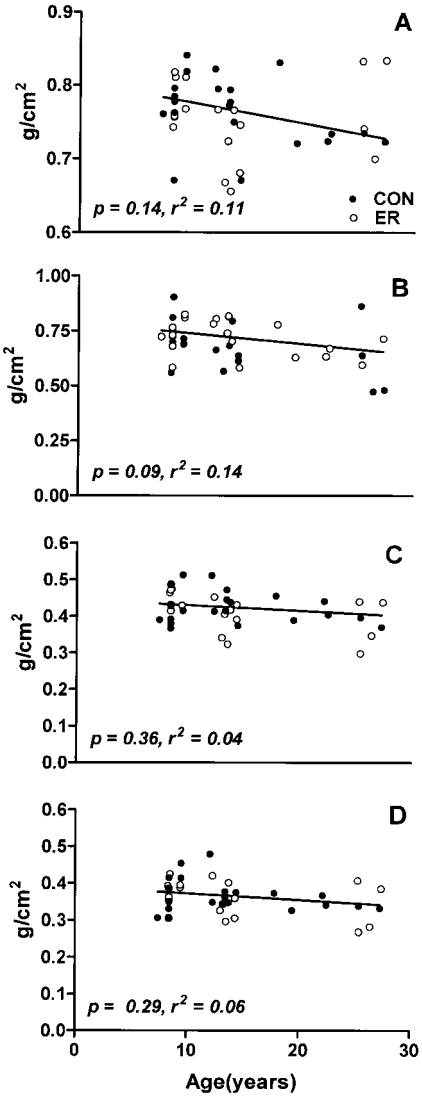

FIGURE 2 Total body (*A*), lumbar spine (*B*), mid radius (*C*) and distal radius (*D*) bone mineral density (BMD) in control (CON, $n = 21$) and 30% energy restricted (ER, $n = 19$) female rhesus monkeys. Each point represents bone mineral density for individual monkeys at the corresponding age. Linear regression analysis revealed no significant cross-sectional effects of age on BMC. ER did not affect BMD at any site. Data were collected after 6 y of ER.

DISCUSSION

Previous studies of the effects of ER on skeletal and reproductive health have suggested a possible negative influence on bone mass (11,12,14,15) and strength (9,13) and an alteration in menstrual cycling (17,20,21). The present study is the first to assess markers of skeletal and reproductive health in female rhesus monkeys. We report here that chronic (6 y) ER initiated at various ages and life stages does not significantly alter bone mass (BMC and BMD) in female monkeys at any of the four skeletal sites examined. In addition, ER did not alter biochemical measures of skeletal metabolism and did not disrupt menstrual cycling or reproductive hormone levels. These findings suggest that ER has no significant negative impact on skeletal or reproductive health in sexually mature, premenopausal or perimenopausal female rhesus monkeys. In addition, ER did not appear to retard or reverse certain age-associated changes in reproductive indices.

TABLE 4

Effects of 30% energy restriction (ER), age and body weight on bone mineral density and content in control (CON) and ER female rhesus monkeys[1]

	Diet (CON vs. ER)		Age, *y*		Body weight, *kg*	
	Regression coefficient	*P*-value	Regression coefficient	*P*-value	Regression coefficient	*P*-value
Bone mineral density, *g/cm*[2]						
Total body	−0.154	0.01	−0.002	0.09	0.026	0.007
Lumbar spine (L2–4)	−0.010	0.74	−0.006	0.006	0.023	0.01
Mid radius	0.002	0.90	−0.003	0.02	0.010	0.04
Distal radius	0.005	0.71	−0.002	0.05	0.011	0.01
Bone mineral content, *g*						
Total body	2.241	0.83	−1.302	0.10	20.726	<0.0001
Lumbar spine (L2–4)	−0.101	0.83	−0.040	0.26	0.409	0.008
Mid radius[2]	−0.060	0.50	−0.008	0.27	0.061	0.04
Distal radius[2]	0.016	0.75	−0.004	0.35	0.061	0.0004

[1] Data presented are results of ANCOVA in which the primary independent variable was diet, with age and body weight added as covariates.
[2] Data were transformed using a Box-Cox procedure (see text) CON, $n = 21$; ER, $n = 19$.

We observed a significant effect of age ($P < 0.05$) on BMD at the lumbar spine and radius sites, suggesting that bone mass at these sites was reduced in older females. We also observed significant age effects on serum estradiol (decrease, $P < 0.05$) and FSH (increase, $P < 0.02$). These findings are consistent with previous studies in female rhesus monkeys. For example, Colman et al. (37) reported an age-related loss of bone mass concomitant with reduced serum estradiol and increased FSH concentrations in a study of 15 aged female rhesus monkeys. Similar hormonal changes in aged females have been reported in other studies (38,39). Last, the observed reduction in the total number and proportion of normal cycles is consistent with previous reports (38,39,47,48) and, when combined with bone mass and hormonal data, suggest that monkeys in the present study exhibited age-appropriate changes in bone mass and reproductive hormones.

In contrast to previous reports in rats on 40% ER (11,12,14,15) and male rhesus monkeys on 30% ER (42), measures of bone mass were not reduced in female monkeys on 30% ER compared with CON monkeys. Several possibilities may explain this lack of agreement. Differences in nutrient intake among the studies seem an unlikely explanation because the diet used in the study of male rhesus monkeys was identical to the diet used in this study. It is also unlikely that differences in nutrient intake influenced the current findings. If indeed differences in nutrient intake between CON and ER monkeys were physiologically relevant, we would have expected significant differences in bone mass to be apparent. Instead, we observed no effect of ER on bone mass. It is possible that this lack of agreement is due to either the duration of ER or the age of the animals. Monkeys in the present study were studied after 6 y of ER compared with 11 y in the study of male monkeys (42). In addition, both the mean and maximum ages of the male monkeys were greater than those of the females in this study. It is also possible that there is a gender-specific response to ER. Although ER studies have been conducted in female rats and mice and are under way in rhesus monkeys (44), studies specifically designed to examine

TABLE 5

Relationship between body composition and bone mineral density and content in control (CON) and 30% energy-restricted (ER) female rhesus monkeys[1]

	Diet (CON vs. ER)		Lean mass, *kg*		Fat mass, *kg*	
	Regression coefficient	*P*-value	Regression coefficient	*P*-value	Regression coefficient	*P*-value
Bone mineral density, *g/cm*[2]						
Total body	0.1	0.52	0.04	0.0004	0.02	0.79
Lumbar spine (L2–4)	−0.01	0.65	0.06	0.02	−0.01	0.66
Mid radius	−0.0004	0.98	0.03	0.01	−0.01	0.28
Distal radius	0.003	0.83	0.03	0.002	−0.01	0.31
Bone mineral content, *g*						
Total body	0.13	0.98	44.60	<0.0001	−0.76	0.90
Lumbar spine (L2–4)	−0.15	0.73	1.0	0.005	−0.13	0.67
Mid radius[2]	−0.08	0.32	0.28	<0.0001	−0.13	0.01
Distal radius[2]	0.003	0.86	0.09	<0.0001	−0.03	0.03

[1] Values presented are results of ANCOVA in which diet was the primary independent variable, with lean and fat mass added as covariates.
[2] Data were transformed using a Box-Cox procedure (see text). CON, $n = 21$; ER, $n = 19$.

TABLE 6

Effects of 30% energy restriction (ER) and age on indices of bone metabolism in control (CON) and ER female rhesus monkeys[1]

	Diet			Age	
Index[2]	CON	ER	*P*-value	Regression coefficient	*P*-value
Osteocalcin, *nmol/L*	1.68 ± 0.11	1.82 ± 0.15	0.45	0.015	0.30
DPD,[2] *nmol/mmol creatinine*	1.55 ± 0.11	1.86 ± 0.19	0.16	−0.057	0.72
PYD,[2] *nmol/mmol creatinine,*	3.07 ± 0.08	3.28 ± 0.13	0.15	0.466	0.26
PTH, *pmol/L*	0.162 ± 0.01	0.148 ± 0.01	0.11	−0.482	0.66
25 (OH)D, *nmol/L*	126.25 ± 7.83	134.17 ± 9.07	0.54	−2.54	0.01
$1,25(OH)_2$-D,[2] *pmol/L*	344.16 ± 20.40	375.12 ± 47.76	0.54	−5.088	0.21

[1] Data for diet are presented as mean ±SEM. CON, $n = 21$; ER, $n = 19$.
[2] DPD, deoxypyridinoline; 25(OH)-D, hydroxyvitamin D; $1,25(OH)_2$-D, 1,25-dihydroxyvitamin D; PTH, parathyroid hormone; PYD, pyridinoline.

differences in male and female responsiveness to ER have not been conducted. Some have suggested that males and females may respond differently to nutritional stress (49,50). As such, possible gender differences in the response to the type of ER regimen used in this study may have contributed to our observation that unlike males, there were no significant effects of ER on bone mass in females. The ongoing longitudinal studies in our laboratory involving both male and female rhesus monkeys offer a unique opportunity to more fully examine this question.

In agreement with previous rat and primate studies (5), we observed that female monkeys on ER exhibited significant changes in body composition compared with CON monkeys. Specifically, ER significantly lowered both body weight and body fat. ANCOVA revealed that body weight was a strong predictor of bone mass at all skeletal sites measured. Among the major body compartments, lean body mass, which was not altered by ER in these monkeys, emerged as the strongest predictor of bone mass. This is in agreement with studies in premenopausal women (51–55) as well as findings from our study in male rhesus monkeys (42). The lack of an effect of ER on lean and bone mass in the present study suggests that these variables may indeed be related. However, Sanderson et al. (11) reported that reductions in bone mass observed in 40% ER rats were related to changes in body weight in those animals. Clearly, additional studies are needed to further explore the relationship between ER-induced changes in body composition and bone mass.

Because chronic ER had been shown to reduce bone mass in rats and in male rhesus monkeys, we believed it important to assess the effect of ER on biochemical markers of calcium homeostasis and skeletal metabolism. We did not observe an effect of 30% ER on serum levels of PTH, 25(OH)D or $1,25(OH)_2$-D. These findings are in agreement with our studies in male rhesus monkeys (42) and some rat studies (11). The work of Kalu et al. (10,14,15) showed that 40% ER prevented an age-related development of renal disease and resulting hyperparathyroidism but that vitamin D concentrations were unaffected by ER. Serum phosphorous and calcium concentrations were also unaltered in the present study (data not shown). Markers of bone formation (osteocalcin) and resorption (PYD and DPD) were likewise unaltered by the diet. A short-term (6–9 wk) ER (40%) regimen in rats reportedly increased bone turnover but did not alter PTH or calcium levels (12). The present findings and previous reports in male monkeys (42) suggest that chronic ER does not alter calcium homeostasis or bone turnover in rhesus monkeys.

We observed that older monkeys in both the CON and ER groups exhibited reductions in the number of menstrual cycles

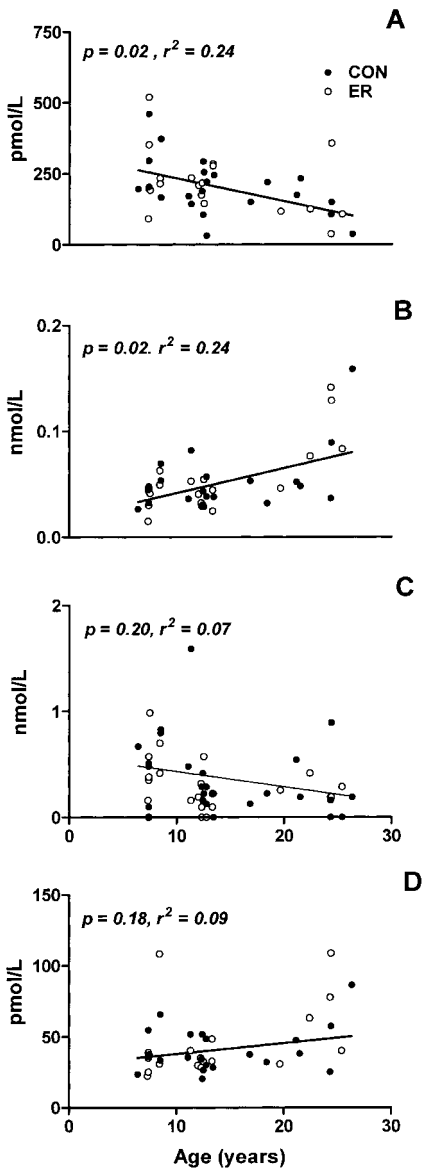

FIGURE 3 Serum estradiol (*A*), follicle-stimulating hormone (FSH: *B*), progesterone (*C*) and luteinizing hormone (LH: *D*) concentrations in control (CON, $n = 21$) and 30% energy-restricted (ER, $n = 19$) female rhesus monkeys. Each point represents biochemical data for individual monkeys at the corresponding age. Data were collected after 6 y of ER. Linear regression revealed significant effects of age on estradiol and FSH concentrations ($P = 0.02$). ER did not affect reproductive hormone concentrations.

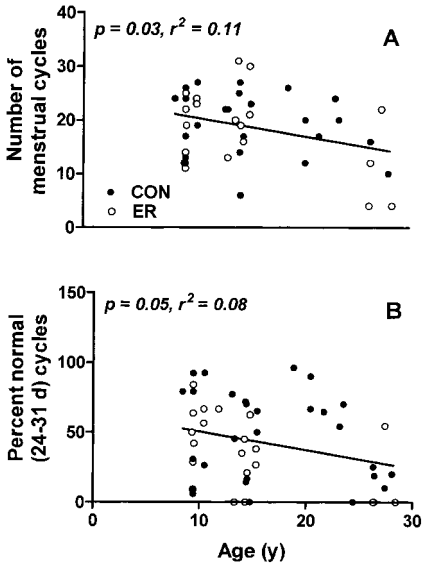

FIGURE 4 Total number (*A*) and percent normal (24–31 d: *B*) menstrual cycles in control (CON, $n = 21$) and 30% energy-restricted (ER, $n = 19$) female rhesus monkeys over 2 y. Each point represents data for individual monkeys. Linear regression analysis revealed that both total number and percent normal menstrual cycles declined with age. ER did not affect menstrual cycling ($P > 0.05$).

and the number of normal length (24–31d) cycles. This suggests that older monkeys in both groups were beginning to experience menstrual difficulty, as has been reported before menopause in both humans and rhesus monkeys (38,39). The finding that ER monkeys did not differ significantly from CON monkeys at any age suggests that this level of restriction (30%) did not alter menstrual cycling. In contrast, rat studies have reported that moderate ER delayed the onset of persistent estrous and reduced serum levels of progesterone (16–19). The effects of ER on FSH and estradiol levels in rats differ depending on the age at which hormone levels were measured (17,18). Two possible explanations for the lack of agreement between rat and monkey findings are readily apparent. First, the ER paradigm used in monkeys uses a more moderate degree of restriction (30%) than that typically used in rats (40%). Another possible explanation relates to species differences in reproductive cycles. Rhesus monkeys exhibit a prolonged (24–31 d) menstrual cycle that is very similar to the cycle in humans. In contrast, the rat estrous cycle is much shorter (4–5 d) and is characterized by more frequent peaks in estradiol.

This study is the first to examine the effects of chronic ER (30%), a nutritional intervention that extends the life span and slows aging in many physiological systems in rodents, on skeletal and reproductive health in female rhesus monkeys. In contrast to previous studies in rats and in male rhesus monkeys, ER does not have a negative impact on bone mass, biochemical indices of skeletal metabolism, menstrual cycling or reproductive hormones in premenopausal monkeys. Furthermore, ER did not retard age-related changes in skeletal or reproductive indices. An assessment of the impact of ER on menopausal and postmenopausal changes must await future studies.

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