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## Calorie Restriction and Bone Health in Young, Overweight

### Individuals

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### Abstract

**Background**—Calorie restriction (CR) is promoted to increase longevity, yet this regimen could lead to bone loss and fracture and therefore affect quality of life.

**Methods**—Forty-six individuals were randomized to 4 groups for 6 months: (1) healthy diet (control group); (2) 25% CR from baseline energy requirements (CR group); (3) 25% energy deficit by a combination of CR and increased aerobic exercise (CR+EX group); and (4) low-calorie diet (890 kcal/d; goal, 15% weight loss) followed by weight maintenance (LCD group). Bone mineral density (total body and hip by dual-energy x-ray absorptiometry) and serum bone markers (bone-specific alkaline phosphatase, osteocalcin, cross-linked C-telopeptide of type I collagen, and cross-linked N-telopeptide of type I collagen) were measured at baseline and after 6 months.

**Results**—Mean $\pm$  SE body weight was reduced by -1.0%  $\pm$  1.1% (control), -10.4%  $\pm$  0.9% (CR), -10.0% $\pm$ 0.8% (CR+EX), and -13.9% $\pm$ 0.7% (LCD). Compared with the control group, none of the groups showed any change in bone mineral density for total body or hip. Bone resorption by serum cross-linked C-telopeptide of type I collagen was increased in all 3 intervention groups, with the largest change observed in the LCD group (CR, 23% $\pm$ 10%; CR+EX, 22% $\pm$ 9%; and LCD, 74% $\pm$ 16% vs control, 4% $\pm$ 10%). Serum levels of cross-linked N-telopeptide of type I collagen were also increased in the LCD group. With regard to bone formation, bone alkaline phosphatase levels were decreased in the CR group (-23% $\pm$ 10%) but were unchanged in the CR+EX, LCD, and control groups.

**Conclusions**—Moderate CR, with or without exercise, that preserves calcium intake for 6 months leads to large changes in body composition without significant bone loss in young adults. Longer

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studies with assessments of bone architecture are needed to confirm that CR nutrientdense diets have no deleterious effect on bone health.

Calorie restriction (CR), a dietary intervention that is low in calories but maintains proper nutrition, is the only intervention known to consistently decrease the biologic rate of aging and increase average and maximal life span. The first randomized controlled trial of CR (Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy [CALERIE]) is being conducted in nonobese men and women. In addition to testing the hypothesis that CR reduces biological aging, assessing the safety of prolonged CR on health, including bone health, is also a primary objective.

Bone mass declines progressively with age in both sexes,<sup>1</sup> and it is well known that conditions of chronic energy deficiency impair bone mineral accrual and bone turnover. Furthermore, weight loss in obese individuals is associated with bone loss.<sup>2</sup> The Washington University CALERIE study recently reported that a 20% reduction in energy intake sustained for 12 months in middle-aged, nonobese adults can significantly reduce bone mass.<sup>3</sup> The reduction in bone mineral density (BMD) (approximately 1.5% overall) at the lumbar spine, total hip, femoral neck, and intertrochanter was correlated with weight loss in the CR group.<sup>3</sup>

The CALERIE study at Pennington was a 6-month study in which 3 different methods of CR were tested in young, nonobese adults. With regard to aging, we found that 2 of the 3 biomarkers for longevity—fasting insulin concentrations and core body temperature—were reduced with CR, while the levels of dehydroepiandrosterone sulfate were unchanged.<sup>4</sup> Also, the metabolic rate was reduced,<sup>4</sup> as were risk factors for type 2 diabetes, including insulin sensitivity, visceral fat, and intrahepatic lipid.<sup>5</sup> Relevant to bone, body mass was reduced by at least 10% in the intervention groups owing to significant losses of both fat mass and fat-free mass.<sup>6</sup> Furthermore, there were significant reductions in fasting concentrations triiodothyronine<sup>4</sup> and leptin, providing possible avenues through which bone turnover could be altered. The aims of this analysis were to determine whether (1) CR with adequate nutrition is associated with changes in bone mass and/or bone turnover markers in young adults; (2) changes in bone mass and/or turnover are less pronounced when the same energy deficit is achieved by combining CR with structured aerobic exercise (CR+EX); and (3) changes in bone mass and/or turnover can be explained by changes in body composition or bone trophic factors such as insulin, triiodothyronine, and leptin.

### METHODS

### SUBJECTS

Forty-six healthy, overweight men and women completed this study (Table 1). As previously reported, participants were excluded if they smoked; exercised more than twice per week; were pregnant, lactating; or postmenopausal; or had a personal history of obesity (body mass index [calculated as weight in kilograms divided by height in meters squared] never>32), cardiovascular disease, diabetes, or regular use of medications (except birth control). The Pennington Center institutional review board, Baton Rouge, Louisiana, and the Data Safety Monitoring Board of CALERIE approved the study. Subjects provided written informed consent.

### STUDY DESIGN

Participants were randomized into 1 of 4 groups for 24 weeks: (1) healthy weight maintenance diet (control group); (2) 25% CR from baseline energy requirements (CR group); (3) 12.5% CR and 12.5% increase in energy expenditure through structured aerobic exercise (CR+EX group); and (4) low-calorie diet (890 kcal/d; goal, 15% weight loss) followed by weight

maintenance (LCD group). Study outcomes were assessed during a 5-day inpatient stay at baseline and during week 24.

### **ENERGY PRESCRIPTION**

The level of energy intake during the intervention was determined for each individual from an assessment of total daily energy expenditure (using doubly labeled water). This weight maintenance calorie target was then slightly modified according to changes in body weight during 2 weeks of controlled feeding.<sup>4</sup>

### DIETS

All diets were based on the American Heart Association guidelines (30% fat, 15% protein, and 55% carbohydrates) and provided the recommended daily intake for all essential vitamins and minerals. During weeks 1 through 12 and 22 through 24 of the intervention, participants consumed only foods prepared by our metabolic kitchen. During weeks 13 through 22, participants self-selected a diet based on their individual calorie target. Multivitamin and mineral supplements (including calcium) were not permitted.

Dietary intake was estimated from 7-day food records completed at baseline (2 times) and at weeks 4, 10, 14, 16, 18, 20, and 22 of the intervention. Participants received extensive training during screening, including a video, written materials, and feedback from a registered dietician. Inability to complete the food record was an exclusion criterion. The behavioral intervention included weekly training for recording food intake using a weight management system (HMR Calorie System; Health Management Resources, Boston, Massachusetts) and photographic assessment of calorie estimation.<sup>7</sup> Food records were reviewed weekly, and immediate feedback on the completeness of the record was provided. The records were analyzed for daily calorie and macronutrient content using the Moore Extended Nutrient (MENu) database, which was developed at our center.

### EXERCISE

Except for participants in the CR+EX group, participants were not permitted to modify their physical activity pattern. Individuals in the CR+EX group increased their energy expenditure by 12.5% above baseline by undergoing supervised aerobic exercise 5 d/wk. The exercise time necessary to expend the 12.5% calorie target was determined for each individual by indirect calorimetry (V-max; Sensormedics, Yorba Linda, California), and exercise sessions were monitored by heart rate (Polar S-610; Polar Beat, Port Washington, New York).

### **BEHAVIORAL INTERVENTION**

Starting at baseline, participants attended weekly meetings that were conducted not only to teach subjects how to adhere to the diet and exercise plans but also to boost motivation and morale and to comply with the interventions during the outpatient part of the study.

### **BODY COMPOSITION AND BONE MASS**

Body weight was determined by the mean of 2 consecutive measurements that were obtained in the morning after a 12-hour fast and corrected for the weight of a hospital gown. Certified technicians who were blinded to the treatment assignments used dual-energy x-ray absorptiometry (QDR 4500A; Hologic Inc, Bedford, Massachusetts) to measure whole-body fat mass and fat-free mass, total body and right hip BMD, and bone mineral content (BMC). Three assessments were made during baseline (days 0, 14, and 28), and 2 assessments were made at month 6. Intraindividual variability of bone assessments was evaluated from triplicate scans in 46 individuals 14 days apart. The coefficients of variation from the whole-body scans were 0.4%, 0.2%, and 0.5% for BMC, BMD, and bone area, respectively, and 1.1%, 0.5%, and 0.9% for the hip scans (total hip, trochanter, neck of femur, and intertrochanter line).

### FASTING SERUM BONE MARKERS

Fasting blood samples were processed immediately, and aliquots were stored at -80°C. As one bone marker may be more sensitive than another for measuring the response to the intervention, <sup>8</sup> we selected 2 serum markers of bone resorption— cross-linked C-telopeptide of type I collagen (Serum Crosslaps; Osteometer, Hawthorne, California) and cross-linked N-telopeptide of type I collagen (Osteomark, Princeton, New Jersey)—and 2 serum markers of bone formation—bone-specific alkaline phosphatase (Alkphase-B; Metra Biosystems Inc, Mountain View, California) and osteocalcin (Diagnostic Systems Laboratories, Webster, Texas). All samples from the same participant were analyzed in duplicate within the same assay. The technician was blinded to group assignment. Using the quality-control samples provided with each assay kit, the interassay and intra-assay coefficients of variation were 5.4% and 11.6%, respectively, for cross-linked C-telopeptide of type I collagen; 3.3% and 5.0% (low control) and 1.9% and 2.2% (high control) for bone alkaline phosphatase; and 5.2% and 12.4% (low control) and 12.6% and 13.4% (high control) for osteocalcin. All determinations were within the limits of detection of the assay.

### STATISTICAL ANALYSIS

A commercially available statistical software package (Version 9.1; SAS Institute Inc, Cary, North Carolina) was used for analyses. Data are expressed as mean $\pm$ SE, with the level of significance set at *P*<.05. At baseline, group, sex, and age effects were tested using a mixed-model analysis of variance (ANOVA). The changes and percent changes from baseline to month 6 were computed for all variables, and ANOVA was performed on the change score to determine differences between groups, with the baseline value and sex as covariates in the model. A linear regression at baseline (n=46×3 scans) was used to generate equations for predicting BMD and BMC from fat-free mass, fat mass, sex, and age. Predicted values for BMD and BMC were calculated at month 6 from the actual values for fat-free mass, fat mass, sex, and age. Differences between the measured and predicted values were tested by ANOVA. The statistical significance for multiple comparisons was adjusted to control for type I errors (Tukey-Kramer method). Relationships between percent changes in BMC, BMD, and biochemical markers of bone turnover vs percent changes in body mass or composition and fasting concentrations of insulin, triiodothyronine, and leptin were determined by Pearson or Spearman correlation coefficients or step-wise regression where appropriate.

### RESULTS

There was an equal distribution of men and women within the 4 groups, and no group differences were observed at baseline for anthropometric, body composition, or bone characteristics (Table 1). At baseline, BMC for the total body and hip was positively associated with body weight ( $r^2$ =0.53 for total body and  $r^2$ =0.44 for total hip; P<.001) and fat-free mass ( $r^2$ =0.66 for total body and  $r^2$ =0.71 for total hip; P<.001).

The nutrient intakes provided during weight maintenance at baseline and prescribed for CR are summarized in Table 2. By design, there was a reduction in caloric intake for each intervention group. During the second half of the intervention, when participants prepared their own meals at home, 7-day diet records indicated good compliance with the prescribed caloric content of the diets (Table 2). Meals prepared by the metabolic kitchen exceeded all the recommended daily allowances for all essential vitamins and minerals. Calcium intake during ad libitum feeding at baseline was 867±135 mg/d in the CR group, 884±218 mg/d in the CR

+EX group, 883±112 mg/d in the LCD group, and 837±87mg/d in the control group. During the self-selected feeding, calcium intake was 898±51 mg/d in the CR group, 1055±89 mg/d in the CR+EX group, 956±69 mg/d in the LCD group, and 1235±118 mg/d in the control group.

As previously reported,<sup>4,6</sup> after 6 months the body weight (Figure 1) was reduced in the CR group (-10.4±0.9%), the CR+EX group (-10.0%±0.8%), and the LCD group (-13.9%±0.7%) compared with the control group, and each intervention group had significant losses of fat mass (CR, -24%±3%; CR+EX, -25%±3%; and LCD, -32%±3%) and fat-free mass (CR, -5%±1%; CR+EX, -3%±1%; and LCD, -6%±1%). Fasting insulin concentrations were significantly reduced in all 3 calorie-restricted groups at month 6 compared with baseline and the control group (CR, -29%±6%; CR+EX, -20%±12%; LCD, -18%±8%; and control, 0%±8%; treatment effect, *P*<.03). Similarly, fasting leptin (CR, -39%±10%; CR+EX, -52%±8%; LCD, -54%±5%; and control, -2%±8%; treatment effect, *P*<.005) and triiodothyronine (CR, -6%±3%; CR+EX, -12%±3%; LCD, -14% ± 3%; and control, 4% ± 3%; treatment effect, *P*<.001) concentrations were reduced in all intervention groups compared with baseline and the control group.

Compared with the control group, no significant effect of treatment was observed for the changes in BMD (Figure 2) and BMC assessed for total body and right hip. In the CR group, total-body BMD increased significantly from baseline by  $1.7\% \pm 0.7\%$  (*P*=.001) but was not different from that in the control group (*P*=.12). To determine whether the changes in BMC and BMD at month 6 were expected based on individual changes in body composition (fat-free mass and fat mass), we compared the actual BMC and BMD measured by dual-energy x-ray absorptiometry with the BMC and BMD derived from the prediction equation at baseline. For both BMD and BMC, there was no significant difference between the actual value measured by dual-energy x-ray absorptiometry and the value derived from the regression model in any group (BMD results shown as actual vs predicted: CR,  $1.17\pm0.1$  g/cm<sup>2</sup> vs  $1.12\pm0.03$  g/cm<sup>2</sup>; CR+EX,  $1.09\pm0.06$  g/cm<sup>2</sup> vs  $1.12\pm0.04$  g/cm<sup>2</sup>).

The level of serum cross-linked N-telopeptide of type I collagen was increased by 31% from baseline to month 6 in the LCD group (P<.001), whereas cross-linked C-telopeptide of type I collagen was increased from baseline in all intervention groups (Figure 3). Bone alkaline phosphatase was reduced by 16% in the CR group, and osteocalcin was increased by 35% in the LCD group (Figure 3). No treatment effect was observed for the changes in any of the bone markers. Changes in serum bone markers were not correlated with changes in bone mass measurements. The changes in insulin, triiodothyronine, or leptin were not correlated with the percent changes of bone resorption or formation markers. The increases in cross-linked C-telopeptide of type I collagen, which occurred with each intervention, were best explained by reductions in weight ( $r^2$ =0.48, P<.001). There was no significant effect of sex in any of the correlation analyses.

### COMMENT

The first studies of CR in humans are currently under way to test the hypothesis that CR slows the rate of aging and affects markers of longevity. Essential to these studies is the parallel evaluation of changes in clinical end points related to impaired health. Bone health is especially important because CR is already being practiced by many individuals, most of whom are normal weight at the initiation of the lifestyle change.<sup>9</sup> In the present study, with tightly controlled assessments of individual energy requirements at baseline and individualized calorie prescriptions for 6 months, we observed significant reductions in body mass, fat mass, and fatfree mass and important metabolic regulators of bone, including leptin, triiodothyronine, and insulin. Compared with a weight-maintained control group, the other groups in our study showed no significant changes in BMC or BMD after 6 months of CR, although some changes

in serum bone markers were observed within groups. The strengths of our study include the randomized, controlled, parallel-group design; the comprehensive assessment of both energy intake and energy expenditure; and the high level of our participants' compliance with the intervention. A limitation in the interpretation of the findings is the capability of dual-energy x-ray absorptiometry to detect changes in bone over 6 months, especially when body weight is drastically changing.

Importantly, there are published studies that have observed changes in BMD and/or BMC with similar interventions that have been shorter than 6 months.<sup>2,3,10,11</sup> For example, Villareal et al<sup>3</sup> measured BMD every 3 months for 12 months in a group of middle-aged overweight men and women (postmenopausal) undergoing CR. A significant reduction in BMD at the hip was observed at the first time point (month 3) and, interestingly, remained lower but stable for the remaining of the study. Similarly, when comparing 12 weeks of diet or diet plus exercise on bone in postmenopausal women, Svendsen et al<sup>11</sup> found a significant reduction in BMD at the lumbar spine. Therefore, it would appear that, at least in older populations, changes in bone mass with CR can be detected by dual-energy x-ray absorptiometry in a time frame that is shorter than 6 months.

Most investigators appreciate the inherent problems with dual-energy x-ray absorptiometry when measuring BMD changes during weight loss. Because of an underestimate of bone area and an overestimate of BMC, the dual-energy x-ray absorptiometer that we used in this study has been shown to inflate BMD with weight and fat loss.<sup>12</sup> As shown in Figure 2A and B, all 3 groups demonstrated a trend toward an increase in BMD after 6 months; however, none of the changes were different from those in the control group. The comparisons of changes in BMD between the control group (no weight loss) and the 3 intervention groups could therefore be susceptible to a type I error owing to these artifacts. Statistical comparisons across the 3 intervention groups, however, should be relatively free of this statistical error because the groups lost a similar amount of weight (approximately 10% to 13%).

To our knowledge, only 1 other study has reported the impact of CR diets with optimal nutrition on bone.<sup>3</sup> The Washington University CALERIE group reported a significant reduction in BMD (lumbar spine, total hip, femoral neck, and intertrochanter) in nonobese men and women aged 50 through 60 years after 3 months of a CR diet.<sup>3</sup> Interestingly, BMD was not reduced by aerobic exercise despite similar weight loss (approximately 10%). It appears that exercise has a protective effect in this group of individuals who are susceptible to bone loss (postmenopausal women). Such effect is in conflict with the current report. It is possible that differences in age and hormonal status could explain the disparity. In the study by Villareal and colleagues,<sup>3</sup> the participants were 12 years older and all the women were postmenopausal. The findings of similar studies comparing a diet-only group with a diet-plus-exercise group (or an exercise group) are conflicting with regard to changes in bone mass. Svendsen and colleagues,<sup>11</sup> who also matched the energy deficit (1000 kcal/d) between the 2 types of intervention in obese individuals, found equivalent reductions in body weight (approximately 12%) and total body BMD with and without exercise. The same result was observed when dieting alone was compared with dieting with resistance training.<sup>13</sup> The same weight loss (approximately 18%) between the groups produced an equivalent reduction in BMC. Therefore, there is no consensus that one kind of intervention for weight loss is superior to another when the maintenance of bone mass is a concern.

While controlled prospective studies that have tested the impact of weight loss or dieting on bone are not directly CR studies, they have generated mixed findings. Collectively, and despite large demographic variability in the study populations and study designs, reductions in body weight greater than 10% are associated with a 1% to 2% reduction in bone mass.<sup>11,14,15</sup> It appears that low-calorie diets that induce rapid weight loss over a short period have the worst

outcome for bone. When weight loss exceeds 20 kg within 3 to 4 months, bone mass is reduced by 3% to 13%.<sup>10,13,16</sup> It is difficult for investigators to determine whether the bone loss is simply a physiological adaptation to the new weight or composition or whether the bone loss is larger than expected on the basis of weight loss. In an attempt to explore this phenomenon, we developed a linear regression model of whole-body BMC and BMD at baseline from fat-free mass, fat mass, sex, and age. When using this regression model to predict BMC or BMD at month 6 for the new body composition of each individual, one can test whether the measured value is statistically different from the value derived from the regression equation. There was no difference between the predicted and measured BMC and BMD at 6 months within each group, and no treatment group was different from the control group. In this population of younger adults, the coefficients from the cross-sectional baseline data suggest that reductions in fat mass—the main depot where weight loss occurred—should have only a modest effect on BMC and BMD (data not shown). The most significant coefficient in the model was fat-free mass, which only marginally decreased (approximately 4%) after 6 months of intervention.

The mechanisms that are cited most often for loss of bone mass with weight loss therapies are the loss of mechanical loading associated with the reduced body mass<sup>17</sup>; changes in the endogenous sex steroid milieu,<sup>18</sup> especially in women; and a reduction in calcium intake,<sup>19</sup> which often occurs with dietary restriction. In a recent study of moderate CR in young obese women, Shapses et al<sup>20</sup> found that bone resorption was increased when calcium intake was reduced and that calcium supplementation (1000 mg/d) could prevent or even increase bone mass. Maintaining calcium intake at normal levels was also important for preventing bone loss in overweight women, with a 7% weight loss over 6 months.<sup>21</sup> Participants in our study were not provided with vitamin or mineral supplements, but the meals that were prepared by the metabolic kitchen during the first 12 weeks and during the testing periods contained more than the daily recommended allowances for essential vitamins and minerals. Self-reported food records indicated that calcium intake was between 900 and 1200 mg/d on average, without a change during the intervention, which probably explains the maintenance of bone health despite a 10% to 14% weight loss.

Hormonal changes that occur with weight loss in obese individuals could also play a role in bone metabolism. For example, leptin has both direct and indirect effects on bone. When directly administered, leptin exerts a stimulatory effect on osteoblast differentiation and on bone growth,<sup>22</sup> while it exerts an inhibitory effect on bone formation when it is administered in the central nervous system.<sup>23</sup> In our study, leptin concentrations were significantly reduced in the 3 intervention groups. Insulin, a regulator of osteoblast growth, has also been reported to be a main determinant of BMD in large cohorts of men and women,<sup>24,25</sup> and a high bone mass is common in hyperinsulinemic states. In addition to the direct effects of insulin on osteoblasts, high levels of insulin lead to an increase in free androgen and estrogen concentrations, thereby altering sex hormone balance. Changes in the hormonal milieu with weight reduction could therefore lead to altered bone turnover. Despite large changes in leptin and insulin, we saw no changes in bone mass or bone turnover markers.

In our study of young (mean±SE age, 37±1 years) overweight but otherwise healthy volunteers, 3 individually prescribed CR interventions did not induce unhealthy changes in total body or hip BMC or density after 6 months. Our data do not support the notion that extreme weight loss (>10%) over short periods (3 months) has a worse prognosis on bone health than gradual weight loss achieved over 6 months by moderate CR with or without aerobic exercise. We speculate that in young individuals undergoing CR, minor adjustments in bone occur as a normal physiological adaptation to the reduced body mass. Further studies of longer duration are warranted and should include an assessment of bone architecture to ensure that bone quality is preserved with weight loss.

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### Figure 1.

Effect of different modes of calorie restriction (CR) on weight as previously shown<sup>4,6</sup> (A) and body composition changes (fat mass and fat-free mass) (B) after 24 weeks. The study participants were randomized into 1 of 4 groups for 24 weeks: (1) healthy weight maintenance diet (control group); (2) 25% CR from baseline energy requirements (CR group); (3) 12.5% CR and 12.5% increase in energy expenditure through structured aerobic exercise (CR+EX group); and (4) low-calorie diet (890 kcal/d; goal, 15% weight loss) followed by weight maintenance (LCD group).



### Figure 2.

Changes in bone mineral density (BMD) (A and B), bone mineral content (BMC) (C and D), and bone area (E and F) across the whole body and the total hip areas after 6 months of calorie restriction (CR). Each box plot shows the distribution of BMD from the 25th to the 75th percentile. The line inside each box plot represents the median. The whiskers reflect the interval between the 10th and the 90th percentiles. The line of identity is also shown. The asterisk indicates significant within-group difference (P<.05). The study participants were randomized into 1 of 4 groups for 24 weeks: (1) healthy weight maintenance diet (control group); (2) 25% CR from baseline energy requirements (CR group); (3) 12.5% CR and 12.5% increase in energy

expenditure through structured aerobic exercise (CR+EX group); and (4) low-calorie diet (890 kcal/d; goal, 15% weight loss) followed by weight maintenance (LCD group).



### Figure 3.

Changes in markers of bone formation (bone alkaline phosphatase [A] and osteocalcin [B]) and bone resorption (serum cross-linked N-telopeptide of type I collagen [sNTx] [C] and serum cross-linked C-telopeptide of type I collagen [sCTx] [D]) after 6 months of calorie restriction (CR). Each box plot shows the distribution of bone mineral density from the 25th to the 75th percentile. The line inside each box plot represents the median. The whiskers reflect the interval between the 10th and the 90th percentiles. The line of identity is also shown. Asterisks indicate significant within-group difference (P<.05). The study participants were randomized into 1 of 4 groups for 24 weeks: (1) healthy weight maintenance diet (control group); (2) 25% CR from baseline energy requirements (CR group); (3) 12.5% CR and 12.5% increase in energy

expenditure through structured aerobic exercise (CR+EX group); and (4) low-calorie diet (890 kcal/d; goal, 15% weight loss) followed by weight maintenance (LCD group). BCE indicates bone collagen equivalents.

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Characteristic	Control (n = 11)	CR (n = 12)	$\mathbf{CR} + \mathbf{EX} (\mathbf{n} = 12)$	LCD(n = 11)
Anthropometric				
Sex, male/female	5/6	9/9	5/7	4/7
Age, y	$37 \pm 2$	$39 \pm 2$	$36 \pm 2$	$39 \pm 2$
Weight, kg	$81.8 \pm 2.8$	$81.0 \pm 3.3$	$82.0 \pm 3.0$	$81.0 \pm 3.3$
BMI	$27.3 \pm 0.5$	$27.8\pm0.5$	$27.5\pm0.5$	$27.5\pm0.5$
Body composition				
Body fat, %	$31 \pm 2$	$31 \pm 2$	$33 \pm 2$	$33 \pm 2$
Fat mass, kg	$25.5 \pm 1.2$	$24.9\pm1.8$	$26.4 \pm 1.7$	$26.5 \pm 1.9$
Fat-free mass, kg	$56.8 \pm 3.1$	$56.3 \pm 3.5$	$55.6 \pm 3.5$	$54.6 \pm 3.4$
Bone mass				
Total body BMD, g/cm <sup>2</sup>	$1.12 \pm 0.02$	$1.15\pm0.03$	$1.08 \pm 0.02$	$1.12 \pm 0.02$
Total body BMC, g	$2439 \pm 93$	$2475 \pm 121$	$2360 \pm 78$	$2411 \pm 102$
Total hip BMD, g/cm <sup>2</sup>	$0.99\pm0.03$	$1.07\pm0.06$	$0.95\pm0.02$	$0.98\pm0.05$
Total hip BMC, g	$34 \pm 2$	$40 \pm 4$	$34 \pm 3$	$35 \pm 3$
Bone turnover markers				
Formation				
Osteocalcin, ng/mL	$14.1 \pm 1.2$	$14.8 \pm 1.0$	$17.3 \pm 1.4$	$13.9 \pm 1.1$
Bone alkaline phosphatase, U/L	$20.0 \pm 2.4$	$26.1\pm11.6$	$23.8\pm3.0$	$18.8 \pm 2.0$
Resorption				
sNTx, nM BCE/L	$23.6 \pm 1.6$	$24.7 \pm 1.6$	$25.8\pm1.8$	$23.7 \pm 1.7$
sCTx, ng/mL	$0.5\pm0.2$	$0.6\pm0.4$	$0.6\pm0.1$	$0.5\pm0.1$
Abbreviations: BCE, bone collagen equivalents	; BMC, bone mineral content; BMI, body	mass index (calculated as weight i	in kilograms divided by height in meter	rs squared); BMD, bone mineral
density; CR, calorie restriction; sCTx, serum cr	oss-linked C-telopeptide of type 1 collage	en; sNTx, serum cross-linked N-tel	opeptide of type 1 collagen.	

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<sup>*a*</sup>The study participants were randomized into 1 of 4 groups for 24 weeks: (1) healthy weight maintenance diet (control group); (2) 25% CR from baseline energy requirements (CR group); (3) 12.5% CR and 12.5% increase in energy expenditure through structured aerobic exercise (CR + EX group); and (4) low-calorie diet (890 kcal/d; goal, 15% weight loss) followed by weight maintenance (LCD group). No group differences were evident at baseline. Values other than sex are presented as mean  $\pm$  SE.

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# Energy Content of the Study Diets<sup>6</sup>

		Mean ± SE (Range), kcal/d	
Group	Baseline Diet <sup>b</sup>	Prescribed Diet <sup>c</sup>	Self-reported Diet <sup>d</sup>
Control	2873 ± 151 (2200-3600)	2873 ± 151 (2200-3600)	2623 ± 179 (1549-3072)
CR	$2800 \pm 158 \; (2200 - 3600)$	$2063 \pm 126 \ (1550-2700)$	$2061 \pm 112 \ (1523 - 2643)$
CR + EX	$2642 \pm 136 \ (2000-3500)$	$2238 \pm 131 \ (1550-3000)$	$2114 \pm 119 (1494-2764)$
LCD	$2755 \pm 130 \ (2200-3300)$	$2091 \pm 124 \ (1650{-}3000)$	$2056 \pm 129 \ (1594-3450)$
Abbreviations: CR, calori	e restriction; LCD, low-calorie diet.		

CR and 12.5% increase in energy expenditure through structured aerobic exercise (CR + EX group); and (4) low-calorie diet (890 kcal/d; goal, 15% weight loss) followed by weight maintenance (LCD <sup>a</sup>The study participants were randomized into 1 of 4 groups for 24 weeks: (1) healthy weight maintenance diet (control group); (2) 25% CR from baseline energy requirements (CR group); (3) 12.5% group). No group differences were evident at baseline.

b. The energy intake at baseline was calculated from two 14-day doubly labeled water assessments of daily energy expenditure and weight fluctuations during 14 days of controlled feeding.

<sup>c</sup>. The amount of energy prescribed during the 6-month intervention. The diet was prepared by the metabolic kitchen for months 0 through 3. Breakfast and dinner were consumed at the center, and lunch and an evening snack were packaged for takeout.

<sup>d</sup>During months 4 through 6, participants were required to follow their prescribed intervention diet. The data shown are the averages of 7-day diet records collected at weeks 14, 16, 18, 20, and 22.