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Effect of 6-Month Calorie Restriction on Biomarkers of Longevity, Metabolic Adaptation, and Oxidative Stress in Overweight Individuals

A Randomized Controlled Trial

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PROLONGED CALORIE RESTRICTION increases life span in rodents and other shorter-lived species.¹ Whether this occurs in longer-lived species is unknown, although the effect of prolonged calorie restriction in nonhuman primates is under investigation. One hypothesis to explain the antiaging effects of calorie restriction is reduced energy expenditure with a consequent reduction in the production of reactive oxygen species (ROS).^{2,3} However, other metabolic effects associated with calorie restriction, including alterations in insulin sensitivity and signaling,

Context Prolonged calorie restriction increases life span in rodents. Whether prolonged calorie restriction affects biomarkers of longevity or markers of oxidative stress, or reduces metabolic rate beyond that expected from reduced metabolic mass, has not been investigated in humans.

Objective To examine the effects of 6 months of calorie restriction, with or without exercise, in overweight, nonobese (body mass index, 25 to <30) men and women.

Design, Setting, and Participants Randomized controlled trial of healthy, sedentary men and women (N=48) conducted between March 2002 and August 2004 at a research center in Baton Rouge, La.

Intervention Participants were randomized to 1 of 4 groups for 6 months: control (weight maintenance diet); calorie restriction (25% calorie restriction of baseline energy requirements); calorie restriction with exercise (12.5% calorie restriction plus 12.5% increase in energy expenditure by structured exercise); very low-calorie diet (890 kcal/d until 15% weight reduction, followed by a weight maintenance diet).

Main Outcome Measures Body composition; dehydroepiandrosterone sulfate (DHEAS), glucose, and insulin levels; protein carbonyls; DNA damage; 24-hour energy expenditure; and core body temperature.

Results Mean (SEM) weight change at 6 months in the 4 groups was as follows: controls, -1.0% (1.1%); calorie restriction, -10.4% (0.9%); calorie restriction with exercise, -10.0% (0.8%); and very low-calorie diet, -13.9% (0.7%). At 6 months, fasting insulin levels were significantly reduced from baseline in the intervention groups (all $P < .01$), whereas DHEAS and glucose levels were unchanged. Core body temperature was reduced in the calorie restriction and calorie restriction with exercise groups (both $P < .05$). After adjustment for changes in body composition, sedentary 24-hour energy expenditure was unchanged in controls, but decreased in the calorie restriction (-135 kcal/d [42 kcal/d]), calorie restriction with exercise (-117 kcal/d [52 kcal/d]), and very low-calorie diet (-125 kcal/d [35 kcal/d]) groups (all $P < .008$). These "metabolic adaptations" (~6% more than expected based on loss of metabolic mass) were statistically different from controls ($P < .05$). Protein carbonyl concentrations were not changed from baseline to month 6 in any group, whereas DNA damage was also reduced from baseline in all intervention groups ($P < .005$).

Conclusions Our findings suggest that 2 biomarkers of longevity (fasting insulin level and body temperature) are decreased by prolonged calorie restriction in humans and support the theory that metabolic rate is reduced beyond the level expected from reduced metabolic body mass. Studies of longer duration are required to determine if calorie restriction attenuates the aging process in humans.

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See also pp 1549 and 1577.

neuroendocrine function, stress response, or a combination of these, may retard aging.⁴

Total energy expenditure is made up of resting energy expenditure (50%-80% of energy), the thermic effect of feeding (~10%), and nonresting energy expenditure (10%-40%).⁵ Whether total energy expenditure is reduced beyond the level expected for a given reduction in the size of the metabolizing mass following calorie restriction is debated. Leibel et al⁶ showed that a 10% weight loss reduced sedentary 24-hour energy intake for weight maintenance between 15% and 20% in obese patients, suggesting that metabolic adaptation occurs in humans. However, the weight loss was achieved quickly with a liquid diet and, with the exception of several normal-weight patients in the study by Leibel et al, the effects of prolonged calorie restriction on energy expenditure in nonobese humans have not been assessed. In rhesus monkeys, resting energy expenditure adjusted for fat-free mass (FFM) and fat mass was lower after 11 years of calorie restriction.⁷ Similarly, total energy expenditure was lower in monkeys following 10 years of weight clamping.⁸ Studies in rodents have proven more controversial with reports of decreased, no change, or increased adjusted energy expenditure in calorie restriction vs ad libitum fed-animals.⁹⁻¹³

One of the most widely accepted theories of aging is the oxidative stress theory, which hypothesizes that oxidative damage produced by ROS accumulates over time, leading to the development of disease such as cancer, aging, and ultimately death.¹⁴ Reactive oxygen species are byproducts of energy metabolism, with 0.2% to 2.0% of oxygen consumption ($\dot{V}O_2$) resulting in ROS formation.^{15,16} Reactive oxygen species attack lipids, proteins, and DNA, generating a number of products that affect normal cell functioning.¹⁷ Studies in rodents subjected to calorie restriction demonstrate a 30% decrease in 8-oxo-7,8-dihydroguanine (8-oxodG) in brain, skeletal

muscle, and heart; similar reductions in carbonyl content in brain and muscle¹⁸⁻²²; and transcriptional patterns that suggest decreased oxidative stress in response to calorie restriction.²³ Rhesus monkeys subjected to calorie restriction exhibit divergent responses in the expression of genes involved in oxidative stress.²⁴

Core body temperature and levels of dehydroepiandrosterone sulfate (DHEAS) and insulin are proposed biomarkers of calorie restriction and longevity in rodents and monkeys.²⁵ Data from the Baltimore Longitudinal Study of Aging support the association between longevity and temperature and insulin and DHEAS levels; men with plasma insulin concentration or oral temperature below the median, and DHEAS levels above the median, live longer.²⁶ Furthermore, in a cross-sectional study that compared individuals following self-imposed nutritionally adequate calorie restriction for 6 years with normal-weight controls, Fontana et al²⁷ found that participants in the calorie restriction group had lower levels of serum glucose, insulin, and markers of atherosclerosis.

The aims of this study were to establish whether prolonged calorie restriction by diet alone or in conjunction with exercise can be successfully implemented in nonobese individuals and to determine the effects of the interventions on established biomarkers of calorie restriction, sedentary energy expenditure, and oxidative damage to DNA and proteins.

METHODS

The Comprehensive Assessment of the Long Term Effects of Reducing Intake of Energy (CALERIE) study is a randomized clinical trial conducted at the Pennington Biomedical Research Center, Baton Rouge, La. The study protocol was approved by the center institutional review board and an independent data and safety monitoring board, and participants provided written informed consent. The study was conducted between March 2002 and August 2004.

Participants

Potential participants (aged <50 years for men and <45 years for women) completed 3 screening visits to ensure physical and psychological health. Assessments of height, weight, and blood pressure were made, and all participants had a chemistry 15 panel, complete blood cell count, and an electrocardiogram. A total of 599 individuals were screened and 551 were excluded (460 were ineligible; 91 withdrew during screening) (FIGURE 1). Race and ethnicity were self-reported. Participants were provided significant monetary compensation both during (at set time points) and on completion of the study. Compensation was calculated and provided in accordance with our institutional review board rules for time and inconvenience. Substantial compensation, along with frequent contact with the study investigators, likely facilitated the excellent retention rate.

Baseline Assessments

Total energy expenditure was measured twice over a 2-week period using doubly labeled water: once while participants followed their usual diet at home, and once while provided a weight maintenance diet. Briefly, participants provided 2 urine samples before being dosed (2.0 g of 10% enriched $H_2^{18}O$ and 0.12 g of 99.9% enriched 2H_2O per kg of estimated total body water), and additional timed samples were taken at 4.5 and 6 hours and 7 and 14 days after dosing. Carbon dioxide output ($\dot{V}CO_2$) and energy expenditure were calculated as previously described.^{28,29} After the second doubly labeled water period, participants attended a 5-day inpatient stay (baseline) where numerous metabolic tests were conducted. Participants repeated the inpatient stay at months 3 and 6.

Intervention

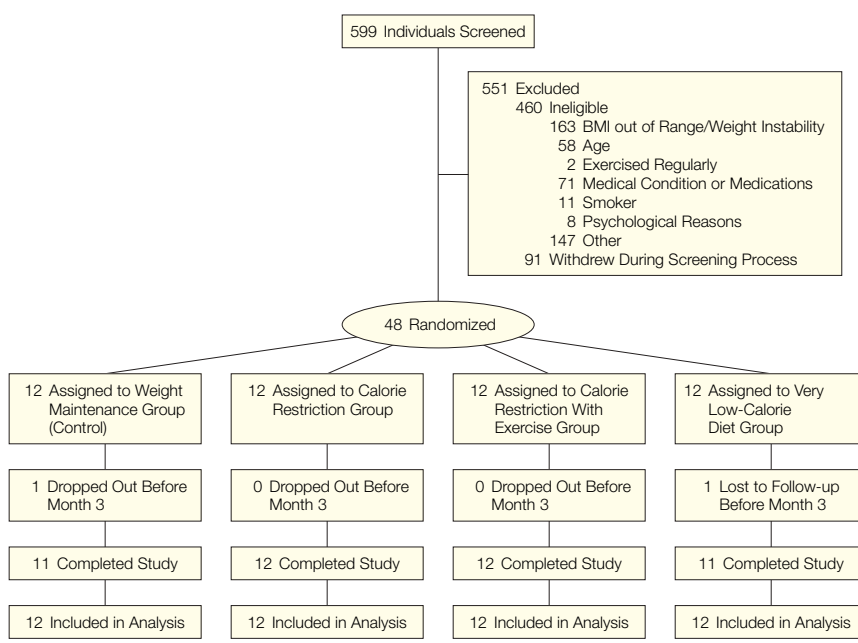
Participants (N=48) were sequentially randomized into 1 of 4 groups for 6 months: (1) control (weight maintenance diet); (2) calorie restriction (25% calorie restriction of baseline energy re-

quirements); (3) calorie restriction with exercise (12.5% calorie restriction plus 12.5% increase in energy expenditure by structured exercise); and (4) very low-calorie diet (very low-calorie diet [890 kcal/d] until 15% reduction in body weight, followed by a weight maintenance diet). Two factors were balanced in study group allocation: sex and 2 categories of body mass index (BMI, calculated as weight in kilograms divided by height in meters squared) (25 to 27.9 and 28 to <30 at screening) according to Pocock and Simon.³⁰ Except for the intervention team, all personnel involved in data collection were blinded to participant information including treatment assignment.

Diets

Energy requirements at baseline were individually calculated from measured energy expenditure. Menus were then prescribed for each participant within 100 kcal of his/her daily target intake. Menus were designed using Moore's Extended Nutrient Database (MEnu 2000, PBRC, Baton Rouge, La) and ProNutra 3.0 (Viocare, Princeton, NJ). Participants were provided with all their food from the last 2 weeks of baseline through week 12. Participants ate 2 meals at the center each weekday, with 1 meal plus snacks packaged for take-out. During weeks 13 through 22, participants self-selected their diet based on individual calorie targets. During weeks 22 through 24, 2 meals per day were provided at the center, with 1 meal and snacks for take-out. All diets (except the very low-calorie diet) were based on American Heart Association recommendations ($\leq 30\%$ fat). The very low-calorie diet was 890 kcal/d (HealthOne, Health and Nutrition Technology, Carmel, Calif) given as 5 shakes containing 75 g of protein, 110 g of carbohydrate, 5 g of fat plus a 10-g bolus of fat per day. Once target weight loss (-15%) was achieved, participants in the very low-calorie diet group were slowly refeed to an energy level that maintained body weight. Generally, target weight was achieved by week 8 in men and by week 11 in women.

Figure 1. Participant Flow in the Trial



Abbreviation: BMI, body mass index.

Behavioral and Exercise Strategies

Participants attended weekly group meetings and initiated a midweek telephone call to report energy intake so that any problems adhering to the protocol were quickly addressed. Cognitive-behavioral techniques were used to foster adherence to diet and exercise prescriptions, including self-monitoring and stimulus control. The Health Management Resources Calorie System (HMR, Boston, Mass) was used to train participants to estimate the caloric content of food.

Participants in the calorie restriction with exercise group increased energy expenditure by 12.5% above resting by undergoing structured exercise (walking, running, cycling) 5 days per week. The mean (SD) target energy cost was 403 (63) kcal per session for women and 569 (118) kcal per session for men. Individual exercise prescriptions were calculated by measuring the oxygen cost (V-Max29 Series, SensorMedics, Yorba Linda, Calif) at 3 levels of the prescribed activity and an equation for estimating energy expenditure was generated. Mean (SD) ex-

ercise duration per session was 53 (11) minutes in women and 45 (14) minutes in men. Participants were required to participate in 3 sessions per week under supervision and wore portable heart rate monitors (Polar S-610, Polar Beat, Port Washington, NY) to assess adherence during unsupervised sessions.

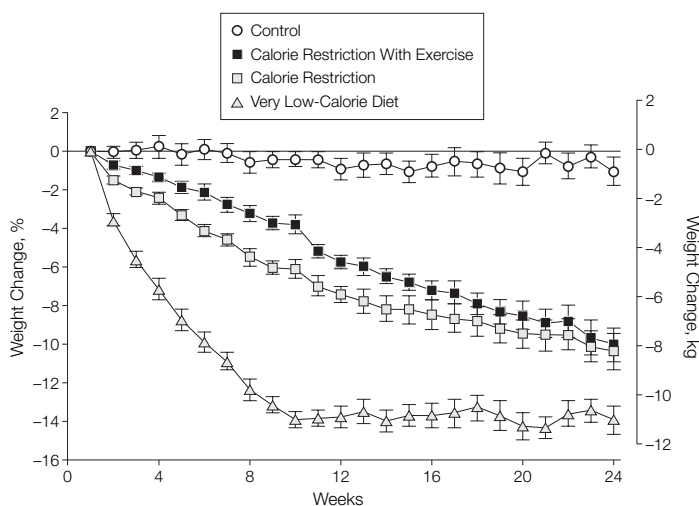
Biochemical Analyses

Fasting serum insulin, DHEAS, thyroxine (T_4), and triiodothyronine (T_3) levels were measured using immunoassays (DPC 2000, Diagnostic Product Corporation, Los Angeles, Calif). Glucose was analyzed using a glucose oxidase electrode (Synchron CX7, Beckman, Brea, Calif). The carbonyl content in proteins was determined using a modified 2,4-dinitrophenylhydrazine assay according to the method of Mates et al.³¹

Metabolic Tests

Weight was measured weekly in a hospital gown following a 12-hour fast after participants had voided. All other metabolic tests were conducted while participants were inpatients at base-

Figure 2. Absolute and Percentage Weight Loss by Group



Initial weight was recorded as the mean of 5 weights measured weekly during the baseline phase. The change in weight over time was significantly different between the control group and the 3 intervention groups ($P < .001$) and between the very low-calorie diet, calorie restriction, and calorie restriction with exercise groups ($P < .001$), but weight loss at week 24 was not significantly different between the very low-calorie diet, calorie restriction, and calorie restriction with exercise groups.

line, month 3, and month 6. Fasting blood samples were taken. Body composition was measured by dual-energy x-ray absorptiometry (QDA 4500A, Hologic, Bedford, Mass). Sedentary energy expenditure (24-hour energy expenditure) was measured over 23 hours in a whole room indirect calorimeter as previously described.³² Three meals and 1 snack were provided at scheduled intervals, and participants were instructed to eat all their food within 30 minutes. Energy expenditure was calculated from $\dot{V}O_2$, $\dot{V}CO_2$, and 24-hour urinary nitrogen excretion³³ and extrapolated to 24 hours. Sleeping energy expenditure was calculated between 2 AM and 5 AM, when motion detectors were reading zero activity.

At baseline, energy intake was matched to measured energy expenditure. However, in keeping with the assigned protocols at months 3 and 6, participants in the calorie restriction group were fed 25% less and participants in the calorie restriction with exercise group were fed 12.5% less than baseline energy expenditure, whereas the participants in the very low-calorie diet

group were fed at a level that matched energy expenditure.

During the metabolic chamber study at baseline and month 6, core body temperature was measured every minute using telemetry pills (CorTemp, HQ Inc, Palmetto, Fla).³⁴ Mean 24-hour, daytime (8 AM-10:30 PM), and nighttime (2 AM-5 AM) temperatures were computed. Due to malfunctions with the monitor or participants passing the pill, complete data were only obtained in 7 of 11 controls, 11 of 12 participants in the calorie restriction group, 8 of 12 participants in the calorie restriction with exercise group, and 9 of 11 participants in the very low-calorie diet group.

DNA Fragmentation

Single cell gel electrophoresis (Comet assay) was conducted according to Deutsch et al.³⁵ Briefly, whole blood cells were suspended in low melting point agarose on commercially available slides (Trevigen, Gaithersburg, Md). The slides were viewed under an ultraviolet microscope (Nikon Microphot FXA, Hamamatsu, Japan [high-resolution 512 lines, Image 1 AT software, FITC 3 fil-

ter]). The extent of DNA damage was determined by calculating the comet tail moment, which is the integrated density in the comet tail multiplied by the distance from the center of the nucleus to the center of mass of the tail, for 25 cells using freely available software (Herbert M. Geller; <http://www2.umdj.edu/~geller/lab/comet.htm>). In 20 individuals measured on 2 consecutive days, the intraclass correlation coefficient of the method was 0.95.

Statistical Analysis

Analyses were carried out for all randomized participants using an intent-to-treat approach without carrying forward the last observation for the 2 dropouts. Data are presented as mean (SEM). SAS version 9.12 (SAS Institute, Cary, NC) was used for analysis. Changes from baseline at month 3 and month 6 were analyzed by a repeated-measures design approach with respect to treatment and time and treatment \times time interactions, with baseline values included as covariates. Data were also analyzed without adjustment for baseline values. Since results by both approaches were similar, we present only the models with adjustment for baseline values. FIGURE 2 illustrates the weight changes in both percent of initial weight and in kilograms; however, all statistical analyses were performed on absolute changes. Linear regression at baseline ($N = 48$) was used to generate equations for predicting energy expenditure, and the predicted values were generated using the equation with measured FFM. Differences between predicted and measured energy expenditure were calculated and analyzed by analysis of variance. A normalizing and variance-stabilizing logarithmic transformation was applied to the calculated tail moments for the comet assay.

Power and sample size calculations were carried out for the primary end point, 24-hour energy expenditure. Sample size was calculated using different levels of baseline 24-hour energy expenditure, assuming a conservative coefficient of variation (7.5%

Table 1. Baseline Screening Characteristics of Individuals Completing the Study (N = 48)

Characteristic	Study Group			
	Control	Calorie Restriction	Calorie Restriction With Exercise	Very Low-Calorie Diet
Sex				
Male	5	6	5	5
Female	7	6	7	7
Race				
White	8	7	7	8
African American	4	4	4	4
Asian or Latino	0	1	1	0
Age, mean (SD) [range], y	37 (7) [27-47]	39 (5) [30-45]	36 (6) [28-45]	38 (8) [26-49]
Weight, mean (SD) [range], kg	81.7 (8.9) [71.1-104.0]	80.9 (11.4) [61.0-101.8]	81.9 (10.5) [65.5-102.4]	82.0 (10.8) [70.4-101.9]
BMI, mean (SD) [range]	27.8 (2.0) [25.1-31.3]	27.8 (1.4) [25.7-30.2]	27.5 (1.6) [25.3-29.8]	27.7 (1.8) [24.7-30.5]
Body fat, mean (SD) [range], %	32.3 (6.6) [22.5-42.9]	31.0 (8.2) [16.9-42.7]	32.6 (7.6) [22.2-43.4]	32.1 (8.1) [20.2-45.4]
Laboratory values, mean (SD) [range]				
Glucose, mg/dL	90 (4) [83-95]	89 (6) [80-101]	92 (6) [82-103]	89 (2) [85-94]
Insulin, μ U/mL	12.3 (3.1) [8.3-17.1]	9.4 (5.1) [4.2-21.0]	9.8 (3.3) [5.7-13.9]	10.8 (2.8) [6.7-15.5]
DHEAS, ng/mL	124 (49) [45-225]	132 (57) [55-234]	161 (88) [51-343]	121 (64) [37-252]
T ₃ , ng/dL	144 (26) [96-175]	139 (23) [97-186]	136 (21) [94-160]	156 (28) [117-212]

Abbreviations: BMI, body mass index, defined as weight in kilograms divided by the square of height in meters; DHEAS, dehydroepiandrosterone sulfate; T₃, triiodothyronine. SI conversions: to convert glucose to mmol/L, multiply by 0.0555; T₃ to nmol/L, multiply by 0.0154.

based on previous chamber studies) and a minimal variability of means. Approximately 12 participants per treatment group were necessary to detect a 15% change in 24-hour energy expenditure from baseline in each group with an 80% power. $P < .05$ was considered statistically significant.

RESULTS

Two individuals withdrew prior to completion of the study: 1 from the control group at week 4 (personal reasons) and 1 from the very low-calorie diet group at week 5 (lost to follow-up) (Figure 1).

Baseline characteristics of the study participants are listed in TABLE 1. Percent weight loss from baseline to month 6 in each group was as follows: controls, -1.0% (1.1%); calorie restriction group, -10.4% (0.9%); calorie restriction group with exercise group, -10.0% (0.8%); and very low-calorie diet group, -13.9% (0.7%) (Figure 2). Fat mass was significantly reduced in all 3 intervention groups compared with baseline and compared with the controls at months 3 and 6 (month 6: calorie restriction group, -24% [3%]; calorie restriction with exercise group, -25% [3%]; very low-calorie diet group,

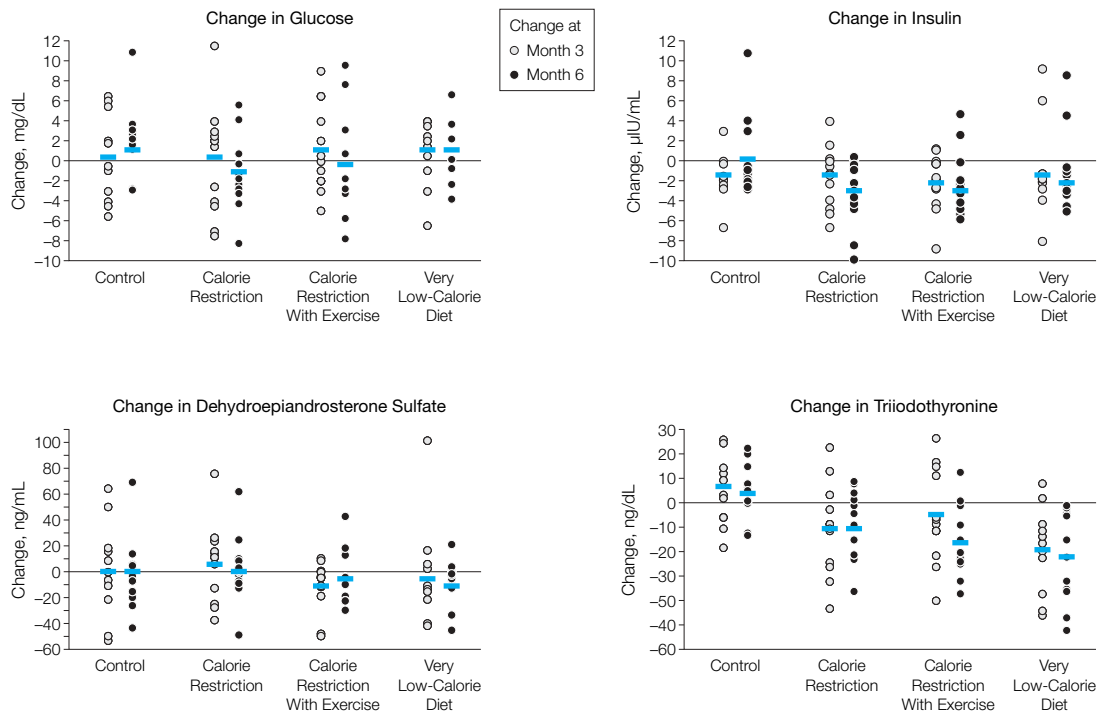
-32% [3%]; $P < .001$). Fat-free mass was significantly reduced in the calorie restriction group (-5% [1%]), the calorie restriction with exercise group (-3% [1%]), and the very low-calorie diet group (-6% [1%]) compared with baseline and controls at month 6 (all $P < .001$).

Fasting insulin levels were significantly reduced from baseline at months 3 and 6 in the calorie restriction and calorie restriction with exercise groups (both $P < .01$ [FIGURE 3]) and at month 6 in all intervention groups (all $P < .01$ [Figure 3]). There were no significant changes in fasting glucose or DHEAS levels in any group. Participants randomized to calorie restriction and calorie restriction with exercise had reduced mean 24-hour core body temperature (FIGURE 4) at month 6. There was no change in core body temperature in the control or very low-calorie diet groups.

Absolute 24-hour energy expenditure and sleeping energy expenditure were significantly reduced from baseline in the calorie restriction, calorie restriction with exercise, and very low-calorie diet groups (all $P < .001$ [TABLE 2]). At baseline, FFM accounted for 86% of the variance in sed-

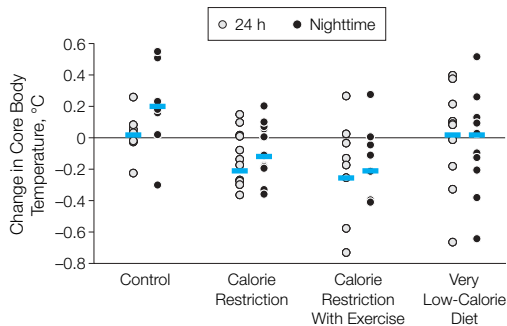
entary 24-hour energy expenditure (24-hour energy expenditure [kcal/d] = $596 + 26.8 \times \text{FFM}$; $r^2 = 0.86$, $P < .001$), whereas fat mass, age, and sex did not statistically account for any additional variance. Compared with predicted 24-hour energy expenditure values, measured daily 24-hour energy expenditure at months 3 and 6 were unchanged in controls and significantly reduced in the calorie restriction, calorie restriction with exercise, and very low-calorie diet groups (Table 2). More specifically, after adjustment for changes in body composition, sedentary 24-hour energy expenditure was unchanged in controls (-18 kcal/d [52 kcal/d]; $P > .05$), but decreased in the calorie restriction (-135 kcal/d [42 kcal/d]), calorie restriction with exercise (-117 kcal/d [52 kcal/d]), and very low-calorie diet (-125 kcal/d [35 kcal/d]) groups (all $P < .008$). These data are shown in Table 2 as actual 24-hour energy expenditure minus predicted energy expenditure. Individual data points at month 6 and the baseline regression line for 24-hour energy expenditure vs FFM are presented in FIGURE 5. When participants from the 3 intervention groups were pooled, adjusted 24-hour energy expenditure values were

Figure 3. Fasting Plasma Glucose, Insulin, Dehydroepiandrosterone Sulfate, and Triiodothyronine Levels at Baseline, Month 3, and Month 6



Fasting insulin was significantly reduced from baseline values at month 3 (not shown) and month 6 in the calorie restriction and calorie restriction with exercise groups. Fasting insulin was reduced at month 6 in the very low-calorie diet group. Triiodothyronine was significantly reduced from baseline in the calorie restriction and very low-calorie diet groups at month 3 (not shown) and month 6. Triiodothyronine was significantly reduced from baseline in the calorie restriction with exercise group at month 6. SI conversion factors: to convert glucose to mmol/L, multiply by 0.0555; triiodothyronine to nmol/L, multiply by 0.0154. Bars indicate mean values.

Figure 4. Change in Core Body Temperature From Baseline to Month 6 Measured Over 23 Hours Inside a Metabolic Chamber Set to a Mean (SD) Temperature of 22.2°C (0.2°C)



Values are for 7 of 11 controls, 11 of 12 participants in the calorie restriction group, 8 of 12 participants in the calorie restriction with exercise group, and 9 of 11 participants in the very low-calorie diet group. Mean 24-hour temperature and nighttime temperature (2 AM-5 AM) are shown. Average 24-hour temperature was significantly reduced from baseline in the calorie restriction and calorie restriction with exercise groups. Nighttime temperature was significantly reduced from baseline in the calorie restriction with exercise group.

statistically lower than controls at months 3 and 6 ($P < .05$).

Since the predicted 24-hour energy expenditure data were derived from

just 48 participants, we also compared the 24-hour energy expenditure data from each group to 865 individuals (510 men; 355 women; mean age, 32

years; mean weight, 88.5 kg) measured in a similar metabolic chamber at the National Institute of Diabetes and Digestive and Kidney Diseases in Phoenix, Ariz.³⁶ Importantly, 24-hour energy expenditure was not different between the reference population and the calorie restriction, calorie restriction with exercise, or very low-calorie diet groups at baseline or at any time point in the controls. However, adjusted 24-hour energy expenditure was significantly lower at months 3 and 6 in the calorie restriction, calorie restriction with exercise, and very low-calorie diet groups (all $P < .01$). Similar to 24-hour energy expenditure, measured sleeping energy expenditure was lower than predicted at months 3 and 6 in the calorie restriction and calorie restriction with exercise groups (Table 2 and Figure 5). There were no significant changes from baseline in the level of spontane-

Table 2. Absolute Energy Expenditures (24-Hour Sedentary and Sleeping) Measured in a Metabolic Chamber At Baseline, Month 3, and Month 6*

Month	Mean (SEM), kcal			Mean (SEM), kcal		
	Actual 24-Hour Energy Expenditure	Predicted Energy Expenditure	P Value	Sleep Energy Expenditure	Predicted Sleep Energy Expenditure	P Value
Control						
Baseline	2129 (102)	2110 (80)		1654 (69)	1642 (60)	
Month 3	2119 (109)	2118 (84)	.89	1642 (92)	1698 (63)	.86
Month 6	2092 (97)	2110 (84)	.38	1513 (37)	1642 (63)	.26
Calorie restriction						
Baseline	2079 (102)	2100 (95)		1600 (88)	1635 (72)	
Month 3	1900 (101)	2048 (91)	.001	1472 (75)	1595 (69)	<.001
Month 6	1899 (101)	2034 (88)	.002	1473 (77)	1585 (66)	.001
Calorie restriction with exercise						
Baseline	2106 (102)	2085 (93)		1615 (78)	1623 (70)	
Month 3	1972 (101)	2057 (89)	.04	1524 (76)	1602 (67)	.02
Month 6	1917 (91)	2034 (86)	.008	1511 (62)	1585 (65)	.03
Very low-calorie diet						
Baseline	2085 (90)	2055 (92)		1658 (78)	1600 (69)	
Month 3	1842 (60)	1965 (82)	.007	1489 (54)	1533 (62)	.13
Month 6	1852 (71)	1977 (87)	.006	1479 (73)	1542 (66)	.19

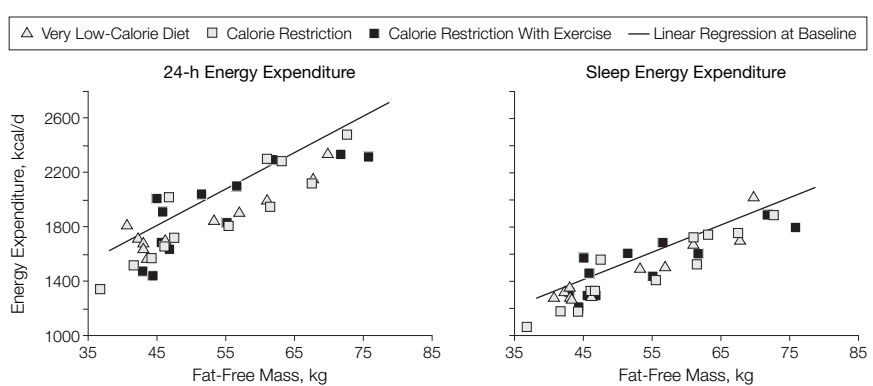
*P values indicate differences between actual vs predicted values. Predicted energy expenditures were calculated as follows: 24-hour energy expenditure = 596 + 26.8 × fat-free mass ($r^2 = 0.86, P < .001$); sleep energy expenditure = 501 + 20.2 × fat-free mass ($r^2 = 0.76, P < .001$). The measured – predicted values for 24-hour energy expenditure and sleep energy expenditure are calculated as the difference between the measured and the predicted values.

ous physical activity or in the thermic effect of food expressed as percentage of energy intake.

Plasma T₃ levels were reduced from baseline in the calorie restriction (−10.2 ng/dL [0.15 nmol/L]) and very low-calorie diet (−18.9 ng/dL [0.29 nmol/L]) groups at month 3 (both $P < .01$) and in the calorie restriction (−8.9 ng/dL [0.13 nmol/L]), calorie restriction with exercise (−4.52 ng/dL [0.07 nmol/L]), and very low-calorie diet (−23.24 ng/dL [0.36 nmol/L]) groups at month 6 (all $P < .02$). A significant treatment effect for plasma T₃ ($P = .001$; Figure 3) with only a tendency for a time effect ($P = .07$) was observed. Similar results were found for change in plasma T₄ level in response to treatment ($P < .05$). When the participants in the 3 treatment groups were combined, we observed significant linear relationships between the change in plasma thyroid hormones and deviations in measured 24-hour energy expenditure from predicted values at month 3 only (T₃: $r = 0.40, P = .006$; T₄: $r = 0.29, P = .05$).

Serum protein carbonyl concentrations were not changed from baseline

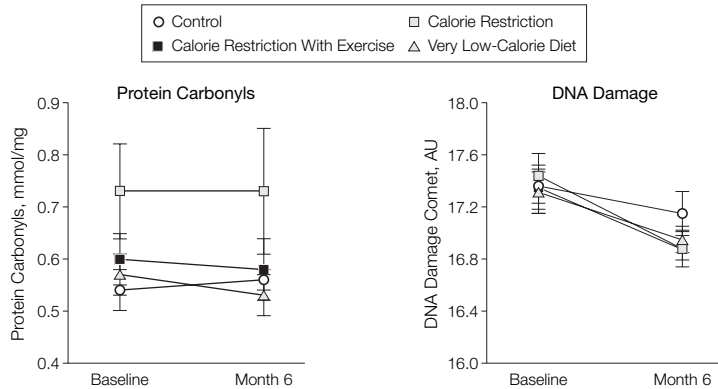
Figure 5. Measured 24-Hour Energy Expenditure, Sleep Energy Expenditure, and Fat-Free Mass at Month 6



Correlation between measured 24-hour energy expenditure and fat-free mass at month 6 (24-hour energy expenditure [kcal/d] = 596 + 26.8 × fat-free mass, $r^2 = 0.86, P < .001$) (left) and measured sleep energy expenditure and fat-free mass at month 6 (sleeping energy expenditure = 501 + 20.2 × fat-free mass, $r^2 = 0.76, P < .001$) (right); fat-free mass was the major determinant of sleep energy expenditure. Regression lines are derived from data at baseline in all participants ($n = 48$) and data markers indicate individual's values at month 6 in the calorie restriction, calorie restriction with exercise, and very low-calorie diet groups.

to month 6 in any group (FIGURE 6). DNA damage was reduced from baseline in the calorie restriction (−0.56 AU [0.11 AU]), calorie restriction with exercise (−0.45 AU [0.12 AU]), and very low-calorie diet (−0.35 AU [0.12 AU]) groups at month 6 (all, $P < .005$), but not in the controls

(Figure 6). This decrease was not statistically different compared with the controls when the 3 treatment groups were combined. We found no significant relationships between the changes in DNA damage and changes in adjusted energy expenditure, fat mass, or body weight.

Figure 6. Fasting Plasma Protein Carbonyls and DNA Damage Measured by the Comet Assay

DNA damage was significantly reduced from baseline in the calorie restriction, calorie restriction with exercise, and very low-calorie diet groups at month 6 (all $P < .005$).

COMMENT

Since the pioneering experiments by McCay and Maynard,³⁷ it has been known that calorie restriction extends life span in rodents and other lower species. However, little is known about the long-term effects of calorie restriction in humans. In the current study, we examined the effects of 6-month calorie restriction on biomarkers of calorie restriction, energy expenditure, and oxidative stress in humans. Our results indicate that prolonged calorie restriction caused: (1) a reversal in 2 of 3 previously reported biomarkers of longevity (fasting insulin level and core body temperature); (2) a metabolic adaptation (decrease in energy expenditure larger than expected on the basis of loss of metabolic mass) associated with lower thyroid hormone concentrations; and (3) a reduction in DNA fragmentation, reflecting less DNA damage.

Numerous biomarkers of calorie restriction have been identified in rodents including temperature, and DHEAS, glucose, and insulin levels. Roth et al²⁶ recently observed that body temperature and insulin and DHEAS levels were also altered in monkeys subjected to calorie restriction, validating their usefulness as biomarkers in longer-lived species. Importantly, they also showed that these parameters were altered in longer-lived men. These find-

ings support the role of these factors as biomarkers of longevity in humans. Similar to the primate model, we observed significantly reduced fasting insulin levels and core body temperatures in the calorie restriction and calorie restriction with exercise groups. However, DHEAS and fasting glucose levels were unchanged by the interventions. Most likely, this study was of insufficient duration to detect changes in DHEAS level, which has been calculated to fall 2% to 4% per year in humans. Fasting glucose level is not consistently altered by prolonged calorie restriction in primates, and thus we question whether fasting glucose level is useful as a biomarker in longer-lived species. On the other hand, Fontana et al²⁷ observed that fasting glucose and insulin levels were substantially reduced in calorie restriction participants who had been following self-prescribed nutritionally adequate calorie restriction diets for 6 years.

Previous studies are inconclusive regarding reductions in metabolic rate following prolonged calorie restriction. In rodents receiving a restricted energy diet for 6 months¹¹ or the entire life span,¹² adjusted resting energy expenditure was not different from controls. In monkeys, adjusted resting energy expenditure was reduced by 60 kcal/d after 11 years of

calorie restriction,⁷ but in previous work, these authors reported no metabolic adaptation after 42 months of calorie restriction.³⁸ Indeed, there are numerous reports in the literature showing either reduced or unchanged adjusted energy expenditure after prolonged calorie restriction in monkeys.^{8,25} In humans, the effects of prolonged, nutrient-dense, calorie-restricted diets in nonobese patients have not been formally investigated. In a starvation study by Keys et al,³⁹ adjusted resting energy expenditure was decreased, which coincided with a reduction in body temperature indicating a real metabolic adaptation.⁴⁰ In the Biosphere 2 experiment, adjusted 24-hour energy expenditure was lower in 5 participants after 2-year calorie restriction, compared with 152 controls.⁴¹ In a study of weight-stable women who had achieved normal body weight using a low-calorie liquid diet, Weinsier et al found that after adjustment for reduced body size, there was no change in resting energy expenditure.⁴²

In this study, we observed a metabolic adaptation over 24 hours in sedentary conditions and during sleep following 6 months of calorie restriction. The metabolic adaptation in the calorie restriction with exercise group was similar to that observed in the calorie restriction group, suggesting that energy deficit rather than calorie restriction itself is driving the decrease in energy expenditure. Importantly, the metabolic adaptations were closely paralleled by a drop in thyroid hormone plasma concentrations confirming the importance of the thyroid pathway as a determinant of energy metabolism.⁴³ Of significance, the metabolic adaptation occurred in the first 3 months of the intervention, with no further adaptation at 6 months, even though weight loss continued in the calorie restriction and calorie restriction with exercise groups.

Metabolic adaptation was also observed over 24 hours but not during sleep in participants in the very low-calorie diet group who were weight

stable when measured at months 3 and 6. Possible explanations for the lack of significant adaptation during sleep in this group include a smaller sample size and the fact that 2 men were regaining weight at month 6. Interestingly, core body temperature and fasting insulin level at month 3 were not changed in this group, despite their having the largest weight loss. Whether metabolic adaptation following calorie restriction persists during weight maintenance remains to be determined in humans.

Spontaneous physical activity and the thermic effect of food were not changed from baseline. However, even if these 2 factors can account for some of the metabolic adaptation, the thermic effect of food accounts for only 10% of daily energy expenditure,⁴⁴ and the cost of activity is already accounted for by a decrease in body weight. Therefore, these 2 factors can only account for a minor part of the metabolic adaptation.

The inverse relationship between increased free radical production, oxidative damage to DNA, and maximum life span has been demonstrated in numerous studies.^{45,46} Caloric restriction in mice down-regulates genes involved in oxidative stress and reduces oxidative damage (8-oxodG), lipid peroxidation, and protein carbonyls.^{18,20,21,23} In nonhuman primates, genes involved in protection against oxidative stress are not altered by calorie restriction, although protein carbonylation is reduced.²² In obese humans, protein carbonylation is also reduced after 4 weeks of calorie restriction.⁴⁷ While we observed no change in protein carbonylation, we are the first to report a significant decline in DNA damage following 6 months of calorie restriction in nonobese men and woman. Contrary to our hypothesis, the reduction in DNA damage was not associated with reduced total or adjusted oxygen consumption in the metabolic chamber.

Considering the lack of correlation between these parameters and the lack of response in protein carbonylation associated with calorie restriction, we are

hesitant to conclude that calorie restriction reduces oxidative stress overall. Clearly, more studies investigating different measures of oxidative stress, such as 24-hour urinary excretion of 8-oxodG, are required. Furthermore, other factors (such as mitochondrial function) may play an important role in oxidative stress. For example, the role of uncoupling proteins in protection against ROS production, independent of changes in proton kinetics and mitochondrial respiration, has recently been demonstrated.⁴⁸

The results of this study show that prolonged calorie restriction by diet or by a combination of diet and exercise was successfully implemented as evidenced by reduced weight, fat mass, fasting serum insulin levels, and core body temperature. This study is unique in that individual energy requirements were carefully measured at baseline and individualized diet goals were determined for each study participant. Furthermore, we observed that "metabolic adaptation" develops in response to energy deficit in nonobese humans at 3 and 6 months leading to reduced $\dot{V}O_2$ per unit of FFM, even after weight stability is achieved. Finally, this study confirms previous findings that calorie restriction results in a decline in DNA damage. However, longer-term studies are required to determine if these effects are sustained and whether they have an effect on human aging.

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Author Contributions: Dr Ravussin had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: DeLany, Larson-Meyer, Volaufova, Greenway, Deutsch, Williamson, Ravussin.

Acquisition of data: Heilbronn, de Jonge, Frisard, DeLany, Larson-Meyer, Nguyen, Martin, Most, Smith, Williamson, Ravussin.

Analysis and interpretation of data: Heilbronn, de Jonge, DeLany, Rood, Volaufova, Deutsch, Williamson, Ravussin.

Drafting of the manuscript: Heilbronn, Rood, Martin, Most, Deutsch, Williamson, Ravussin.

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Statistical analysis: Heilbronn, Volaufova, Ravussin.
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Study supervision: Heilbronn, Frisard, Larson-Meyer, Most, Greenway, Deutsch, Williamson, Ravussin.

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We should be careful to get out of an experience only the wisdom that is in it—and stop there; lest we be like the cat that sits down on a hot stove-lid. She will never sit down on a hot stove-lid again—and that is well; but also she will never sit down on a cold one anymore.

—Mark Twain (1835-1910)

Table. Annual Number of Laparoscopic Cases

Procedure	Years Since Introduction														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Cholecystectomy	16 247	93 464	270 991	363 161	354 565	348 323	331 076	333 600	327 092	316 733	319 793	346 157	351 736	360 844	358 069
Fundoplication	19	184	1613	5299	11 245	13 111	15 802	18 399	23 993	24 761	24 188	18 981	19 042		
Hysterectomy	4838	6181	13 102	38 929	44 852	41 401	42 335	48 578	68 455	60 805	60 733	64 639	69 659	71 977	76 033
Nephrectomy indication															
Cancer	35	236	215	199	283	308	563	532	701	1226	1968	4221	5093		
Benign disease	452	454	573	614	767	898	1261	1055	1947	1662	1896	2823	3388		
Donor	11	4	19	21	40	154	473	449	510	1589	1305	1648	1789		

Critical revision of the manuscript for important intellectual content: Miller, Dunn, Wei, Hollenbeck.

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Study supervision: Wei, Hollenbeck.

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CORRECTIONS

Incorrect Unit of Measure: In the Original Contribution entitled "Effect of 6-Month Calorie Restriction on Biomarkers of Longevity, Metabolic Adaptation, and Oxidative Stress in Overweight Individuals: A Randomized Controlled Trial" published in the April 5, 2006, issue of *JAMA* (2006;295:1539-1548), an incorrect unit of measure was given for dehydroepiandrosterone sulfate (DHEAS). On page 1543 (Table 1) and page 1544 (Figure 3), the unit of measure for DHEAS should be $\mu\text{g/dL}$ (not ng/mL).

Error in Byline: In the Original Contribution entitled "Incidence and Prognostic Implications of Stable Angina Pectoris Among Women and Men" published in the March 22/29, 2006, issue of *JAMA* (2006;295:1404-1411), the byline contained an incorrect academic degree. Alison McCallum should have been listed as having an MBChB, FFPH.

Incorrect Data: In the Original Contribution entitled "Frequency and Effect of Adjuvant Radiation Therapy Among Women With Stage I Endometrial Adenocarcinoma" published in the January 25, 2006, issue of *JAMA* (2006;295:389-397), incorrect data were reported in the "Results" section of the article. On page 391, the sentence "Within the RT cohort, 2551 patients (62.5%) had external beam radiation, 732 (17.9%) had vaginal brachytherapy, and 1078 (26.4%) received a combination of external beam radiation with vaginal brachytherapy" should have read "Within the RT cohort, 2378 patients (58.3%) received external beam radiation, 962 (23.6%) received external beam and brachytherapy radiation, 654 (16.0%) received brachytherapy radiation alone, and for 86 (2.1%) the radiation modality was not specified." The authors verified that this error did not have an impact on the data set or subsequent statistical analyses.

Incorrect Statements on Funding/Support and Role of the Sponsors and Incorrect and Incomplete Financial Disclosures: In the Review entitled "Anti-TNF Antibody Therapy in Rheumatoid Arthritis and the Risk of Serious Infections and Malignancies: Systematic Review and Meta-analysis of Rare Harmful Effects in Randomized Controlled Trials" published in the May 17, 2006, issue of *JAMA* (2006;295:2275-2285), the following errors appeared:

After this issue was printed and mailed, *JAMA* was informed by the authors that information reported on page 2284 of the article was incorrect.

The Funding/Support statement should have read "This study was supported by the Mayo Foundation. Additional data were provided by Abbott and Centocor. Data provided by Abbott were subject to a confidentiality agreement."

The Role of the Sponsors statement should have read "Abbott and Centocor did not have any role in the design and conduct of the study; the collection, management, analysis, and interpretation of the data; or the preparation or approval of the manuscript. The manuscript was sent to Abbott for review prior to submission for publication."

The Financial Disclosures statement should have read: "Dr Bongartz reported that he has given lectures for Abbott as part of seminars for study nurses and received honorarium in the form of a medical textbook for the Internal Medicine library; he received an educational grant from Amgen in February 2006 to perform the same type of analysis of harmful events under anti-TNF treatment for etanercept; and he received the 2005 Fellow's Award of the American College of Rheumatology, which was supported by Amgen."

Dr Matteson reported that he has been a paid consultant for Centocor for work unrelated to this study and has been working with Wyeth and Amgen to perform a similar analysis for etanercept; he has been an Investigator for the American College of Rheumatology, Amgen, Asta, Biogen-IDEC, Burroughs-Wellcome, Centocor, Cypress, Endocyte Inc, Genentech, Hoffmann-LaRoche, Human Genome Sciences, Immunex, Protein Design Laboratories, Nastech, Pharmacia & Upjohn, Schering, Wyeth, and Xoma Corp; he has received grant support from Amgen, Aventis, Centocor/Johnson & Johnson, Genentech, Immunex, Mayo Foundation, Novartis, and the National Institutes of Health; and he has been a consultant for Amgen, BoneandJoint.org, Burroughs-Wellcome, Centocor, Regeneron, Takeda, Upjohn, Watermark Research, and the Vasculitis Foundation."

This correction was published online on May 16, 2006. Because of the nature and extensiveness of this incorrect and incomplete reporting, *JAMA* has requested that the Mayo Clinic College of Medicine conduct an investigation. *JAMA* will publish another correction or clarification once the results of that investigation become available.