Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism[1,2]

Leonie K Heilbronn, Steven R Smith, Corby K Martin, Stephen D Anton, and Eric Ravussin

ABSTRACT

Background: Prolonged dietary restriction increases the life span in rodents. Some evidence suggests that alternate-day fasting may also prolong the life span.

Objective: Our goal was to determine whether alternate-day fasting is a feasible method of dietary restriction in nonobese humans and whether it improves known biomarkers of longevity.

Design: Nonobese subjects (8 men and 8 women) fasted every other day for 22 d. Body weight, body composition, resting metabolic rate (RMR), respiratory quotient (RQ), temperature, fasting serum glucose, insulin, free fatty acids, and ghrelin were assessed at baseline and after 21 d (12-h fast) and 22 d (36-h fast) of alternate-day fasting. Visual analogue scales were used to assess hunger weekly.

Results: Subjects lost 2.5 ± 0.5% of their initial body weight ($P < 0.001$) and 4 ± 1% of their initial fat mass ($P < 0.001$). Hunger increased on the first day of fasting and remained elevated ($P < 0.001$). RMR and RQ did not change significantly from baseline to day 21, but RQ decreased on day 22 ($P < 0.001$), which resulted in an average daily increase in fat oxidation of ≥15 g. Glucose and ghrelin did not change significantly from baseline with alternate-day fasting, whereas fasting insulin decreased 57 ± 4% ($P < 0.001$).

Conclusions: Alternate-day fasting was feasible in nonobese subjects, and fat oxidation increased. However, hunger on fasting days did not decrease, perhaps indicating the unlikelihood of continuing this diet for extended periods of time. Adding one small meal on a fasting day may make this approach to dietary restriction more acceptable. *Am J Clin Nutr* 2005;81:69–73.

KEY WORDS Resting metabolic rate, fat oxidation, insulin, glucose, biomarkers of longevity

INTRODUCTION

Prolonged dietary restriction (DR) is the only proven method of increasing the life span in rodents, flies, yeast, and worms (1). The mechanism or mechanisms by which DR increases life span are unclear, but the effects of DR include reduced metabolic rate, reduced oxidative damage, altered neuroendocrine signaling, and improved insulin sensitivity (2). The effect of prolonged DR on the life span in nonhuman primates is currently being investigated (3–5). Although conclusive results are years away, many improvements in biomarkers of longevity, including reduced core temperature, resting metabolic rate (RMR), dehydroepiandrosterone sulfate, glucose, and insulin, have already been observed. Prolonged DR also alters the expression of many genes from skeletal muscle, brain, and liver, including genes encoding heat shock proteins and uncoupling proteins and genes involved in oxidative damage (6–8). Recent microarray results in mouse liver indicate that there is significant overlap of genes that are up-regulated by short-term starvation and by prolonged DR (9).

Alternate-day fasting may therefore be an alternative to prolonged DR as a method of increasing maximal life span. Goodrick et al (10) found that alternate-day fasting increased median and maximal life span in C57Bl/6 mice when it was introduced at 1.5 and 6 mo of age and increased maximal, but not median, life span in A/J mice. Recently, Anson et al (11) observed that mice fed every other day consumed the same total energy as did ad libitum fed animals and had similar body weights but had reduced glucose and insulin concentrations and increased resistance to endotoxic stress (11).

A pilot study testing the feasibility and effects of long-term DR on biomarkers of longevity in nonobese humans is currently under investigation. This randomized clinical trial named CALERIE (sponsored by the National Institute of Aging) is testing numerous behavioral strategies and diets (ranging from liquid energy to 20–30% DR to increased energy expenditure by physical activity) to determine which of these will prove the most viable in today's "obesogenic" environment. However, the feasibility and efficacy of alternate-day fasting is not being investigated. Given the difficulty that individuals have in estimating energy intake (12–14), alternate-day fasting may prove to be a less complicated method than prolonged DR in humans. Indeed, one study investigated the effects of alternate-day DR for 3 y (15). In that study, the subjects were allowed 1 L of milk and 2–3 pieces of fruit on their energy-restricted day and 9600 kJ/d on the other day. The control group was fed 9600 kJ/d every day. The subjects randomly assigned to alternate-day DR spent less time in the infirmary and had a lower death rate than in the control group (6 versus 13; NS) (16). The present study was undertaken to determine the feasibility of alternate-day fasting in nonobese subjects. In addition, the effects of alternate-day fasting on body weight, RMR, fat oxidation, and biomarkers of longevity were investigated.

[1] From the Pennington Biomedical Research Center, Baton Rouge, LA.

[2] Reprints not available. Address correspondence to E Ravussin, Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA, 70808. E-mail: ravusse@pbrc.edu.

Received May 27, 2004.

Accepted for publication September 2, 2004.

TABLE 1
Baseline characteristics of the participants by sex[1]

	Men ($n = 8$)	Women ($n = 8$)
Age (y)	34 ± 3	30 ± 1
Weight (kg)	80.6 ± 4.4	59.7 ± 1.7[2]
BMI (kg/m^2)	25.2 ± 1.1	22.6 ± 0.6
Fat mass (%)	22 ± 2	25 ± 1
Cholesterol (mmol/L)	4.9 ± 0.4	4.7 ± 0.2
HDL (mmol/L)	1.0 ± 0.1	1.8 ± 0.1[2]
Triacylglycerols (mmol/L)	2.5 ± 0.6	1.1 ± 0.1[2]
Systolic blood pressure (mm Hg)	116 ± 2	104 ± 3[2]
Diastolic blood pressure (mm Hg)	75 ± 3	68 ± 2

[1] All values are $\bar{x}$ ± SEM.
[2] Significantly different from men, $P < 0.01$ (one-factor ANOVA).

SUBJECTS AND METHODS

Subjects

Healthy, nonobese [body mass index (in kg/m^2) range: 20.0–30.0] men ($n = 8$) and women ($n = 8$) aged between 23 and 53 y were recruited (**Table 1**). The subjects had different levels of physical activity: 7 were sedentary, 3 were moderately active (exercised 1–2 times/wk), and 6 were quite active (exercised 4–5 times/wk). Competitive athletes and subjects with type 2 diabetes were excluded. The Institutional Review Board of the Pennington Biomedical Research Center approved the study, and the subjects gave their written informed consent.

Study design

The subjects attended the clinical research center on 2 consecutive days at baseline (days −2 and −1) and on 2 consecutive days after 3 wk of alternate-day fasting following a "feast" day (day 21) and following a "fast" day (day 22). The subjects had therefore fasted 12 h (overnight) on days −2, −1, and 21 and 36 h on day 22. The subjects were instructed to avoid exercise, alcohol, and coffee for ≥24 h before each visit. At each visit, the subjects arrived in the clinic at 0700 and were weighed while wearing a hospital gown. Blood pressure was measured with the subject in a seated position after a 5-min rest, oral temperature was recorded (SureTemp; Welch Allyn Inc, NY), and a fasting blood sample was drawn. RMR was measured for 30 min with a DeltaTrac metabolic monitor (SensorMedics, Yorba Linda, CA) after a 20-min resting period while the subjects were awake in a semirecumbent position. On days −2 and 21, body composition was measured by dual-energy X-ray absorptiometry (QDR 4500; Hologic Inc, Bedford, MA). At baseline and on days 1, 7, 15, and 21 (fasting days), the subjects completed visual analogue scales (VASs) at 1000, 1200, 1400, and 1600 to assess their feelings of hunger, fullness, desire to eat, satisfaction, and prospective food consumption (17). Briefly, the participants were asked to place a mark on a 100-mm line anchored by "not at all" and "extremely" to record subjective levels of hunger or satiety. The VASs were scored by measuring from the left end of the line to the mark in mm, and mean ratings were calculated for each day. At baseline, the subjects also completed the Eating Inventory questionnaire, which assessed dietary restraint (the intent and ability to restrict caloric intake), disinhibition (the tendency to overeat), and hunger (18). The subjects also completed a nine-item self-report questionnaire, which was developed for this study, to assess eating attitudes and behaviors with the use of an 8-point scale. This questionnaire (Eating Behaviors Questionnaire) assessed whether the subjects consider themselves "dieters" who watch what they eat or "big eaters" who tend to eat 1 or 2 large meals per day.

After baseline testing was completed, the subjects fasted from midnight to the subsequent midnight on alternating days for 22 d. On each fasting day, the subjects were allowed to consume energy-free beverages, tea, coffee, and sugar-free gum and were instructed to keep their water intake high. On each feasting day, the subjects were instructed to eat whatever they wished and were informed that double their usual food intake would be required to maintain their usual body weight. The subjects were provided with calibrated digital scales (Tanita, Arlington Heights, IL) to record their morning fasting body weight, urinary sticks to test for the presence of ketones, and a diet diary to record anything that was consumed on the fasting day. On day 20, the subjects were required to fast from 1900 so that a 12-h overnight fast would be completed before testing began the following morning at 0700. They did not break this fast until after their clinic visit on day 22.

Biochemical analytes

Glucose was analyzed by using a glucose oxidase electrode (Syncron CX7; Beckman, Brea, CA). Free fatty acids were measured on a Synchron CX5 by using reagents from Wako (Richmond, VA). β-Hydroxybutyrate was measured on a Synchron CX5 by using reagents from Sigma (St Louis). Insulin was measured by using an immunoassay on a DPC 2000 (Diagnostic Product Corporation, Los Angeles). Ghrelin was measured by using a radioimmunoassay kit from Linco (St Charles, MO).

Statistical analysis

Data are expressed as means ± SEMs. SAS 8.2 (SAS Institute Inc, Cary, NC) and SPSS 11.0.1 (SPSS Inc, Chicago) were used for data analysis. Baseline measures (days −2 and −1) were averaged. Statistics were performed by one- and two-factor repeated-measures analysis of variance. Post hoc analysis was performed with Tukey's tests where necessary. RMR was analyzed by using linear regression to adjust for fat mass and fat-free mass. Correlations were performed with Pearson's correlation coefficient. Significance was set at $P < 0.05$. Fasting insulin values below the detection limit of the assay (<2.0 mU/L) were assigned a value of 1.0 mU/L. Insulin values were log transformed for analysis.

RESULTS

The subjects' characteristics by sex are given in Table 1. On the basis of their self-recorded diet diaries and weight logs (**Figure 1**), the subjects complied with the protocol. Urinary ketones were not useful as a measure of compliance because they were not consistently detected in all subjects (data not shown). On the basis of daily regressed body weights, the subjects lost 2.5 ± 0.5% of their initial body weight. This self-reported weight loss was confirmed by weights measured in the clinic at baseline and on days 21 and 22 ($P < 0.001$). Significant reductions were observed in fat mass ($P < 0.001$) and fat-free mass ($P < 0.05$) after the intervention (Figure 1).

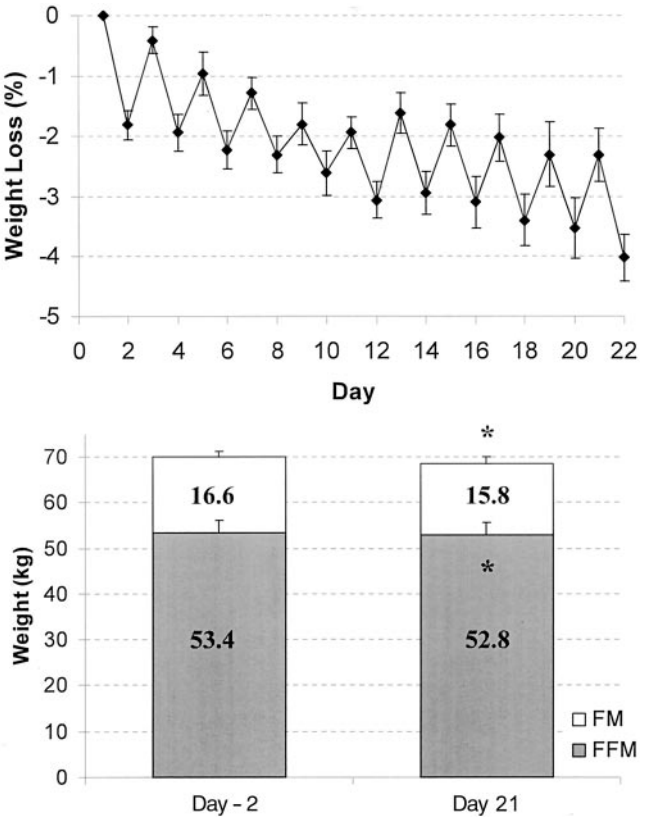

FIGURE 1. Top panel: mean (±SEM) percentage change in daily self-recorded body weight ($n = 16$). Body weight was measured before breaking an overnight fast (odd days) or after 24–36-h fasts (even days). Bottom panel: mean (±SEM) fat mass (FM) and fat-free mass (FFM) by dual-energy X-ray absorptiometry measured after 12-h fasts at baseline (day −2) and on day 21. *Significantly different from baseline, $P < 0.001$ for FM and $P < 0.05$ for FFM (one-factor ANOVA).

On average, the men considered themselves "big eaters," and the women reported that they "watched what they ate." Percentage weight loss did not differ significantly between the men and the women, but weight loss correlated negatively with considering oneself a big eater after adjustment for sex ($r = -0.63$, $P = 0.04$). The dietary restraint and disinhibition scales of the Eating Inventory questionnaire did not significantly predict weight loss.

VASs were completed for all days by only 8 of 16 subjects. First, baseline results were compared with the first day of fasting. As expected, a significant increase was found in feelings of hunger (from 37 ± 5 to 56 ± 4 mm; $P < 0.001$), and a significant decrease was noted in feelings of fullness (from 43 ± 3 to 23 ± 4 mm; $P < 0.001$). However, repeated-measures analysis over time (days 1, 7, 15, and 21) showed no significant changes in the subjects' perception of hunger, thirst, desire to eat, or feelings of satisfaction, although feelings of fullness increased slightly over time ($P < 0.05$).

Temperature (data not shown) and absolute and relative resting metabolic rate (adjusted for fat-free mass and fat mass) were not significantly different from baseline (**Table 2**). Respiratory quotient (RQ) was also not significantly different from baseline at day 21; however, RQ was lower on day 22 ($P < 0.001$; Table 2). More specifically, fat oxidation increased from 64 g/24 h at baseline to 101 g/24 h, and carbohydrate oxidation decreased from 175 to 81 g/24 h. The change in RQ from baseline to day 21 was related to weight loss ($r = -0.76$, $P < 0.001$).

TABLE 2
Resting metabolic rate (RMR), respiratory quotient (RQ), and fat and carbohydrate oxidation measured at baseline and after a fed day (day 21) and a fast day (day 22)[1]

	Baseline	Day 21	Day 22
RMR (kJ/d)	6675 ± 283	6292 ± 268	6329 ± 260
RQ	0.85 ± 0.01	0.86 ± 0.02	0.79 ± 0.01[2]
Fat oxidation (g/24 h)[3]	64 ± 8	54 ± 10	101 ± 9[2]
Carbohydrate oxidation (g/24 h)[3]	175 ± 17	184 ± 24	81 ± 16[2]

[1] All values are $\bar{x}$ ± SEM. Two consecutive days at baseline were averaged for analysis.
[2] Significantly different from baseline, $P < 0.001$ (one-factor repeated-measures ANOVA).
[3] Calculated by assuming that protein oxidation was 15% of RMR.

The women had significantly lower glucose, insulin, free fatty acid, triacylglycerol, and LDL-cholesterol concentrations and significantly higher HDL-cholesterol and ghrelin concentrations than did the men ($P < 0.05$). Fasting glucose was not significantly changed from baseline in the men or the women (**Figure 2**). Fasting insulin was lower on day 22 in both the men and the women ($P < 0.001$), and fasting β-hydroxybutyrate and free fatty acid concentrations were higher on day 22 in both the men and the women (Figure 2; $P < 0.001$). Fasting ghrelin was not significantly altered from baseline on day 21 (results not shown) or day 22 (from 1019 ± 128 to 1063 ± 158 pg/mL in the men and from 1403 ± 63 to 1493 ± 139 pg/mL in the women). Systolic and diastolic blood pressure were not significantly altered by the intervention (data not shown). HDL was elevated from baseline in the women only ($P < 0.001$; data not shown), and triacylglycerol was significantly reduced from baseline in the men only ($P < 0.05$; data not shown).

DISCUSSION

Alternate-day fasting may be an alternative to prolonged DR for increasing the life span (11). In the present study, we report that alternate-day fasting is feasible for short time periods in nonobese subjects. One participant reported feeling lightheaded once, and 4 subjects reported constipation. No subjects withdrew during the study, but many reported feeling irritable on their fasting days, perhaps indicating the unlikelihood of continuing this diet for extended periods of time. The results from the VASs suggest that feelings of fullness may have increased from the first fasting day over the course of the study, but other subjective states related to food intake motivation did not habituate, including hunger. This result contrasts with the results of studies using liquid-based, very-low-energy diets where hunger diminishes despite a marked energy deficit (19). Overall, these results suggest that a prolonged schedule of fasting and feasting would be marred by aversive subjective states (eg, hunger and irritability), which would likely limit the ability of most individuals to sustain this eating pattern.

This is the first study, to our knowledge, to test the effects of alternate-day fasting on body weight and other metabolic variables in humans. Body weight was clearly reduced from baseline after 3 wk of alternate-day fasting, indicating that the subjects were unable to consume enough food on the feasting days to

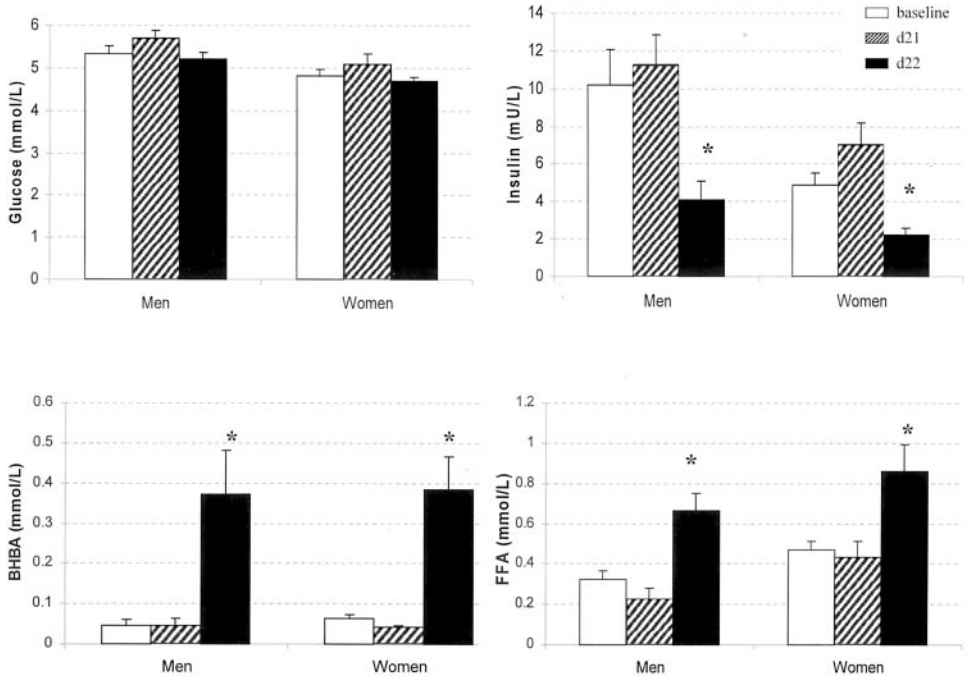

FIGURE 2. Mean (±SEM) fasting glucose, fasting insulin, fasting β-hydroxybutyrate (BHBA), and fasting free fatty acids (FFA) at baseline, day 21, and day 22 in men ($n = 8$) and women ($n = 8$). *Significantly different from baseline, $P < 0.01$ [two-factor (time and sex) repeated-measures ANOVA].

maintain their weight. This is opposite the results observed in rodents, where mice fed every other day maintained their body weight and consumed roughly the same amount of food in 1 d that ad libitum–fed animals consumed over 2 d (11). We hypothesized that the subjects with a self-reported ability to overeat or eat large amounts of food would maintain their body weight, and this hypothesis was supported: considering oneself a "big eater" was negatively associated with weight loss when sex was controlled for by partial correlation. Whether alternate-day fasting would lead to weight loss in obese participants remains unclear. The negative subjective states associated with the study cast doubt on the ability of individuals to voluntarily engage in alternate-day fasting for prolonged periods of time. Altering the clock time that the subjects are asked to fast (eg, from 1900 to 1900) or adding a small meal (10–20% of caloric needs) to the fasting day may make alternate-day fasting more acceptable in all populations.

Ghrelin is a peptide secreted in the gut that is reduced on feeding and has been implicated in the regulation of feeding behavior and energy balance. Obese subjects have lower fasting ghrelin concentrations than do lean subjects (20) but have impaired suppression of plasma ghrelin in response to a meal (21). Furthermore, ghrelin is increased after weight loss in obese subjects (22, 23), perhaps driving the common phenomenon of weight regain after weight loss. In the present study, the women had significantly higher ghrelin concentrations than did the men. This has been reported previously (24) but is not consistently observed (25) and may be related to central adiposity. In contrast with the large increases in reported hunger, plasma ghrelin was unchanged in both the men and the women, even after 36 h of fasting. Studies in rodents have found that 24-h fasts increase plasma ghrelin (26). However, fasting for 72 h did not change plasma ghrelin in lean men (24). The results of these fasting studies in humans call into question the role of ghrelin in the hunger drive and highlight the need for further research in this area.

A hallmark of rodent studies of longevity is reduced fasting glucose and insulin concentrations and increased insulin sensitivity in dietary-restricted animals (27). Reduced fasting insulin has also been associated with increased longevity in humans (27). In the present study, insulin was reduced after a fast day, suggesting improved insulin sensitivity. However, plasma free fatty acids were also elevated after fasting; these elevated concentrations may impair insulin-mediated glucose disposal and the suppression of hepatic glucose production (28). We also found that alternate-day fasting did not significantly change fasting glucose or insulin from baseline after a 12-h fast. This is in contrast with results in mice, in which glucose and insulin concentrations were lower after 14-h fasts than in ad libitum fed–mice or mice fed energy-restricted diets. Thus, humans may need to fast for longer than 12 h for this effect to be observed. Alternatively, this could be due to the already low glucose concentrations of our population or that 3 wk of alternate-day fasting was insufficient to produce this response. The study design may also have affected these results, because the subjects anecdotally reported eating even more than usual on day 20 (knowing they were about to enter a longer than usual fast day).

RMR was not significantly changed after 3 wk of alternate-day fasting. The effects of 36-h fasts on RMR have not been previously reported. Horton and Hill (29) observed no differences in metabolic rate (measured for 12 h in a metabolic chamber after a mixed meal) between overnight or 3-d fasts. We did observe that subjects oxidized more fat on day 22 as evidenced by a reduction in RQ from 0.85 to 0.79. However, RQ was not altered on day 21. This suggests that there were no sustained increases in fat oxidation on fed days. Caution must be exercised when interpreting this result, because the subjects did not consume standardized

diets and RQ is heavily dependent on fat intake and energy balance. However, it is more likely that we underestimated fat oxidation, because the subjects were coming out of positive energy balance and because overall fat oxidation was increased by an average of ≥15 g/d. Furthermore, because weight loss is positively correlated with increased fat oxidation, the results suggest that the subjects with a greater ability to oxidize fat lost more weight. Alternatively, it could be argued that the subjects who had a greater caloric deficit had increased fat oxidation.

In conclusion, alternate-day fasting is feasible in nonobese subjects for short time periods, although unlike rodents, the subjects were unable to maintain their body weight. Furthermore, fat oxidation was increased and translated into fat mass loss. Hunger on fasting days did not habituate over the course of the study, which perhaps indicates the unlikelihood of subjects continuing on this diet for extended periods of time. Whether alternate-day fasting would promote weight loss in an obese population is uncertain.

We acknowledge the clinical research staff for their assistance in performing this study and Julia Volaufova for assistance with the statistical analysis.

LKH, SRS, and ER were involved in developing the study protocol and the experimental design. CKM and SA administered and analyzed the VAS and psychological questionnaires. LKH wrote the draft manuscript with contributions from ER, SRS, and CKM. None of the authors had any financial interests in organizations sponsoring this research.

REFERENCES

1. Weindruch R, Walford RL. The retardation of aging and disease by dietary restriction. Springfield, IL: Charles C Thomas Publisher, 1988.
2. Heilbronn LK, Ravussin E. Calorie restriction and aging: review of the literature and implications for studies in humans. Am J Clin Nutr 2003; 78:361–9.
3. Ingram DK, Cutler RG, Weindruch R, et al. Dietary restriction and aging: the initiation of a primate study. J Gerontol 1990;45:B148–63.
4. Kemnitz JW, Weindruch R, Roecker EB, Crawford K, Kaufman PL, Ershler WB. Dietary restriction of adult male rhesus monkeys: design, methodology, and preliminary findings from the first year of study. J Gerontol 1993;48:B17–26.
5. Hansen BC, Bodkin NL, Ortmeyer HK. Calorie restriction in nonhuman primates: mechanisms of reduced morbidity and mortality. Toxicol Sci 1999;52:56–60.
6. Lee CK, Klopp RG, Weindruch R, Prolla TA. Gene expression profile of aging and its retardation by caloric restriction. Science 1999;285: 1390–3.
7. Lee CK, Weindruch R, Prolla TA. Gene-expression profile of the ageing brain in mice. Nat Genet 2000;25:294–7.
8. Cao SX, Dhahbi JM, Mote PL, Spindler SR. Genomic profiling of short- and long-term caloric restriction effects in the liver of aging mice. Proc Natl Acad Sci U S A 2001;98:10630–5.
9. Bauer M, Hamm AC, Bonaus M, et al. Starvation response in mouse liver shows strong correlation with lifespan prolonging processes. Physiol Genomics 2004;17:230–4.
10. Goodrick CL, Ingram DK, Reynolds MA, Freeman JR, Cider N. Effects of intermittent feeding upon body weight and lifespan in inbred mice: interaction of genotype and age. Mech Aging Dev 1990;55:69–87.
11. Anson RM, Guo Z, de Cabo R, et al. Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake. Proc Natl Acad Sci U S A 2003;100:6216–20.
12. Hill RJ, Davies PS. The validity of self-reported energy intake as determined using the doubly labelled water technique. Br J Nutr 2001;85: 415–30.
13. Heymsfield SB, Darby PC, Muhlheim LS, Gallagher D, Wolper C, Allison DB. The calorie: myth, measurement, and reality. Am J Clin Nutr 1995;62(suppl):1034S–41S.
14. Schoeller DA. Limitations in the assessment of dietary energy intake by self-report. Metabolism 1995;44:18–22.
15. Vallejo EA. La dieta de hambre a dias alternos in la alimentacion de los viejos. Rev Clin Exp 1957;63:25–6 [in Spanish].
16. Stunkard AJ. Nutrition, aging and obesity. In: Rockstein M, Sussman ML, eds. Nutrition, longevity, and aging. New York: Academic Press, 1976:253–84.
17. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes Relat Metab Disord 2000;24:38–48.
18. Stunkard AJ, Messick S. Eating inventory manual (The Psychological Corporation). San Antonio, TX: Harcourt Brace & Company, 1998.
19. Wadden TA, Stunkard AJ, Day SC, Gould RA, Rubin CJ. Less food, less hunger: reports of appetite and symptoms in a controlled study of a protein-sparing modified fast. Int J Obes 1987;11:239–49.
20. Tschöp M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. Diabetes 2001;50:707–9.
21. English PJ, Ghatei MA, Malik IA, Bloom SR, Wilding JPH. Food fails to suppress ghrelin levels in obese humans. J Clin Endocrinol Metab 2002;87:2984.
22. Cummings DE, Weigle DS, Frayo RS, et al. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N Engl J Med 2002; 346:1623–30.
23. Hansen TK, Dall R, Hosoda H, et al. Weight loss increases circulating levels of ghrelin in human obesity. Clin Endocrinol (Oxf) 2002;56: 203–6.
24. Chan JL, Bullen J, Lee JH, Yiannakouris N, Mantzoros CS. Ghrelin levels are not regulated by recombinant leptin administration and/or three days of fasting in healthy subjects. J Clin Endocrinol Metab 2004; 89:335–43.
25. Purnell JQ, Weigle DS, Breen P, Cummings DE. Ghrelin levels correlate with insulin levels, insulin resistance, and high-density lipoprotein cholesterol, but not with gender, menopausal status, or cortisol levels in humans. J Clin Endocrinol Metab 2003;88:5747–52.
26. Beck B, Richy S, Stricker-Krongrad A. Ghrelin and body weight regulation in the obese Zucker rat in relation to feeding state and dark/light cycle. Exp Biol Med (Maywood) 2003;228:1124–31.
27. Roth GS, Lane MA, Ingram DK, et al. Biomarkers of caloric restriction may predict longevity in humans. Science 2002;297:811.
28. Homko CJ, Cheung P, Boden G. Effects of free fatty acids on glucose uptake and utilization in healthy women. Diabetes 2003;52:487–91.
29. Horton TJ, Hill JO. Prolonged fasting significantly changes nutrient oxidation and glucose tolerance after a normal mixed meal. J Appl Physiol 2001;90:155–63.