Physiologic Effects of Lowering Caloric Intake in Nonhuman Primates and Nonobese Humans

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Caloric restriction (CR) reduces the rate of aging and increases life span in all small animal species studied to date, but the effects of CR in humans remain uncertain. This review summarizes current knowledge of the effects of CR in nonhuman primates and humans. The results suggest that CR has a range of beneficial effects in nonhuman primates studied under laboratory conditions, and short-term markers of CR seen in animal models appear to occur in humans subject to CR also. However, the overall benefit of CR in human populations remains to be established, and studies in human populations are needed.

Reducing morbidity and delaying mortality in the elderly population are recognized as major goals of aging research (1,2). In addition to improving the quality of life for elderly individuals, even modest reductions in morbidity would have a major impact on national health care costs, which are disproportionately affected by the number of elderly subjects in the population. Studies in a wide range of nonprimate animal species have demonstrated repeatedly that caloric restriction (CR) significantly reduces the rate of aging and increases life span. In addition, emerging physiologic measures in nonhuman primate species are consistent with previously reported findings in rodents. However, it is not known whether these findings are applicable to humans.

The focus of this review is the physiological effects of CR in humans and nonhuman primates, and related information on morbidity and mortality associations with CR is discussed.

To a large extent, the lack of information on the effects of CR in humans reflects the difficulty of conducting long-term CR studies in human populations, both from the perspective of the ethical issues involved and from methodological considerations. The following background sections highlight available information and discuss information that may be relevant to future projects.

Studies in Nonhuman Primates

CR studies were initiated in nonhuman primates more than 10 years ago because they could provide more controlled data on the effects of CR than would be obtained easily in humans. This summary focuses on findings from the three major studies of CR in primates (3–8). The first and largest of these was begun at the National Institute on Aging (NIA) in 1987 and now involves more than 200 monkeys of various ages and both genders. The second study, begun in 1989 at the University of Wisconsin–Madison (UW), involves about 80 monkeys of both genders. In the NIA study, CR was begun at various stages throughout the rhesus monkey life span (young, 1–2 yr; young adult, 3–5 yr; and older adult, >15 yr). Both of these studies utilize a 30% CR regimen based on either the age- and weight-specific average intake of controls (NIA) or on each individual monkey’s baseline consumption (UW). It is very important to note that control monkeys in these studies are not generally obese, and only a few have become obese over the course of the study. The third study, at the University of Maryland (UMD), evolved from ongoing investigations of obesity and diabetes. That study involves a limited number (8 at the start of the study) of older adult male rhesus monkeys on a weight stabilization protocol that required a reduction in caloric intake of about 35%. In that study a greater proportion of controls were obese, some having as much as 48% body fat. Nonetheless, it is interesting to note that results from the UMD study often show good agreement with those of the NIA and UW studies (9,10). The NIA also has studied effects of CR in a short-lived primate species (squirrel monkeys) whose maximal life span is about 25 years. Preliminary findings suggest that markers such as body weight, body temperature, and serum glucose are marginally reduced in squirrel monkeys on CR (unpublished findings, M.A. Lane).
Physiological findings from the monkey studies show remarkable consistency with rodent data. The remainder of this section discusses findings relevant to body composition, carbohydrate and energy metabolism, lipids, bone and reproductive aging, and disease markers.

**Body Composition**

Young, growing rhesus monkeys placed on CR at the NIA continue to gain body weight, fat, and lean mass, but at a slower rate compared to controls. Upon reaching stable adult weight, all body composition parameters, including lean mass, are reduced in CR monkeys compared to controls. When CR was begun in monkeys that had already reached a stable weight (UW and NIA), studies showed that all body composition parameters are lowered in CR animals compared to controls. In the UW study this was a result of both reductions in CR monkeys and gains in controls. Older monkeys placed on CR at the NIA (>15 yr) exhibited reductions in most parameters. Note that some CR monkeys are able to defend body composition and maintain weight (or even gain a little) for a significant time after CR has been initiated. As demonstrated at the NIA, both male and female monkeys on CR have less fat in the trunk (central) region and that the trunk-to-leg fat ratio is reduced in CR animals compared to control monkeys. In the UW study it was additionally shown that CR monkeys had a lower percentage of body fat in the abdominal regions (9). A short-term study of monkeys on CR (but eating a high cholesterol diet) at Bowman Gray Medical School used CT scanning to show that visceral fat was reduced in CR monkeys as well as a larger amount of fat from other depots (11).

**Carbohydrate and Energy Metabolism**

The NIA, UW, and UMD studies focused on glucoregulation as a potentially major outcome variable. Despite differences in diet composition and body composition between study groups, CR consistently reduces fasting glucose and insulin and improves insulin sensitivity and glucose uptake. Insulin secretory responses (acute and secondary phases) are also significantly attenuated in CR monkeys. These findings are not unexpected when comparing relatively lean CR monkeys to obese controls (>22% fat). However, it is interesting to note that the NIA studies have shown that the same effects were observed comparing CR monkeys to lean controls (12–16% fat). These findings suggest that to some extent the effects of CR on carbohydrate metabolism may be independent of obesity or altered body composition. Short-term CR studies at the NIA further support this notion. It has been shown also that changes in insulin secretion and responsiveness occur very early during the initiation of CR (0–3 months), even though body composition changes are not yet evident at that early stage.

The three studies have also investigated energy metabolism and have reported generally consistent data to those reported in rodents. The initial period of CR is accompanied by a significant reduction in 24-hour energy expenditure (indirect calorimetry and isotopic dilution) on both an absolute basis and when energy expenditure is adjusted for lean body mass. However, after 2–3 years on CR, whole body energy expenditure is either reduced or not changed, depending on the study (12,13).

Other indices of energy metabolism that have been studied include locomotor activity and body temperature. Average locomotor activity (measured by infrared sensors placed on the monkeys’ cages) monitored over a one-week period showed no significant effect of CR on average activity level during the 24-hour period. However, CR monkeys are slightly more active just prior to meal times in the early morning and afternoon. The effects of CR on body temperature were also studied; after about 3 years on CR, rectal body temperature (measured in anesthetized, fasted monkeys) was significantly reduced. Radiotelemetry devices were also utilized to show that, during adaption to CR, body temperature was reduced by about 1.5°C in young, unanesthetized monkeys.

**Serum Lipids and Blood Pressure**

Both the NIA and UW studies have reported that CR reduced serum triglycerides. Generally, total cholesterol shows a consistent, but not always statistically significant, reduction in CR monkeys. This may be due in part to the low cholesterol diets being fed to these monkeys. Total high-density (HDL) and low-density lipoprotein (LDL) cholesterol is similarly unaltered by CR either during the short- or long-term experiments. However, in the UW study, LDL cholesterol particles isolated from CR monkeys are of a lower molecular weight compared to controls and particles from CR monkeys that are depleted of triglyceride. The effect of CR on the various HDL subfractions in the NIA monkeys was examined, and CR was also shown to increase levels of HDL-2B, particularly in young, lean monkeys.

Both systolic and diastolic pressures are consistently lower in CR monkeys compared to controls. However, this effect is consistently statistically significant only in female monkeys. It should be noted that older rhesus monkeys in the NIA study show no evidence of hypertension. Aortic pulse wave velocity (an index of arterial stiffness) was examined in the NIA male rhesus control and CR monkeys and showed that pulse wave velocity was reduced by an average of about 20% in all three age groups.

**Bone and Reproductive Aging**

Dual energy x-ray absorptiometry (DXA) has been used to study bone mineral density (BMD) in both male and female rhesus monkeys at the NIA over the past 5 years. In female monkeys only pre- and perimenopausal females have been studied to date. In males, the oldest cohort contains several animals over 30 years of age. Data are available in both gender groups for young, growing monkeys that had not yet achieved peak bone mass, adult monkeys of a stable weight, and older monkeys that might be expected to show evidence of bone loss or menstral changes. Cortical (mid radius), trabecular (ultra distal radius, lumbar spine), and total body sites were studied. It should be noted that although adequate intakes of calcium and vitamin D are provided in both diet groups, intakes of these nutrients were greater in the ad libitum (AL) than CR animals.
Similarly, bone has been studied in both male and females in the UW study. Bone mass by DXA (total body, lumbar spine, and radius), bone turnover, and lumbar spine osteoarthrits were examined longitudinally. Total bone mass was lower in CR monkeys of both sexes. In the females, CR bone mass was also lower at the lumbar spine and radius sites. Serum turnover markers and calcium and phosphorus concentrations were not different between CR and control groups. In addition, testosterone and follicle-stimulating hormone (FSH) in the males were unchanged. There was less evidence of osteoarthrits in CR males. These findings are interpreted to indicate that lower bone mass in CR animals reflects smaller body size, rather than pathological osteopenia (14,15).

Similar to humans, rhesus monkeys exhibit a slight reduction in BMD at several skeletal sites during aging. Female monkeys on CR exhibit a slight reduction in BMD at the mid- and distal-radius sites as well as in the lumbar spine. This effect is very slight and is statistically significant (main effect of diet) only at the mid-radius. Male monkeys also exhibit slight but significant reductions in BMD in the oldest groups. Similar to our observations in the female monkeys, males on CR generally have lower BMD, but the main effect of diet is significant only at the distal radius. Peak bone mass at any site (achieved at about 8–10 years of age in rhesus monkeys) is also slightly, but not significantly, reduced in CR monkeys compared to controls (NIA study). Similar findings have been observed in the UW colony.

Various markers related to bone turnover, calcium homeostasis, and reproductive hormones have been studied. There is no effect of CR on bone markers (i.e., serum osteocalcin or urinary N-telopeptide cross links of Type I bone collagen) in either male or female monkeys. It also has been observed that CR does not alter serum levels of vitamin D or parathyroid hormone in either sex. Finally, serum calcium and phosphorus levels are unchanged in CR monkeys compared to controls. A 3-year study of menstrual cycling and reproductive hormones was conducted with the NIA female monkeys. Monkeys were observed each day for evidence of menstrual bleeding. At several timepoints during the study, blood samples were collected on day 5 of the cycle for hormone assay. Menstrual cycle length and regularity were also determined. CR had no effect on serum levels of estradiol, progesterone, FSH or luteinizing hormone. CR monkeys in all age groups continued to cycle throughout the study, but menstrual irregularity was apparent in several old monkeys from both control and CR groups. A tendency was observed for older CR monkeys to exhibit more abnormal-length cycles (<24 or >31 days long), but this effect was not significant. Studies of diet-related weight loss in premenopausal women suggest that ovulatory changes including short luteal phase cycles and anovulation within normal cycle intervals will occur rather than loss of menstrual cyclicity (16,17). Subclinical disturbances of ovulation occurring in weight-stable premenopausal women are associated with significant bone loss; this explains over 20% of the one-year change in vertebral cancellous bone density and is likely important (18). However, systematic studies of ovulation and luteal phase length have not yet been performed in any of the CR monkeys and controls.

Summary of Physiological Effects of CR in Nonhuman Primates

The nonhuman primate studies of CR show good agreement for most physiological markers, and findings from these studies are remarkably consistent with rodent data. One potentially negative finding is that BMD is slightly reduced in CR monkeys. However, this cannot currently be attributed to a significant alteration in markers of bone formation or resorption, calcium homeostasis, or reproductive hormones. It is possible that the observed slight reductions in BMD are related to body composition changes in CR monkeys, but they may also relate to subclinical, subtle disturbances of ovulation. Further studies of bone changes and ovulatory characteristics as well as body composition changes are needed.

None of the ongoing nonhuman primate studies have experienced sufficient mortality to permit determination of whether CR extends life span in primates. Preliminary data from the NIA study suggests that death due to cardiovascular disease (5 control and 1 CR) and cancer (3 control and 1 CR) may be reduced by CR. However, these findings are based on a small number of animals and as such they should not be overinterpreted.

Naturally Occurring CR in Human Populations

Effects on Metabolic Rate, Hormonal Parameters, and Morbidity

From the perspective of human biology, caloric restriction is a common nutritional regime in many populations—not, of course, as a matter of choice. The populations affected are often rural populations in the developing world, and so are often subject to poverty, high physical workloads, high infectious and parasitic disease burdens, and a low level of medical care and other social services. An important concern in studies of undernourished individuals is that their diets may be deficient in multiple nutrients, not just energy. For this reason, the information cannot be equated to CR in animals, because a central tenet of the CR field is that the diet must contain adequate amounts of protein, minerals, and vitamins (19). However, at the very least, appreciation of the consequences of CR in undernourished populations can help identify potential advantages and disadvantages of human CR conducted under more nutritionally favorable circumstances.

Many of the consequences of naturally occurring caloric restriction in human populations are well known. In particular, chronic energy deficiency is associated with short stature and late reproductive maturation (20). Recent data have indicated that both women and men who mature under conditions of low energy intake have lower levels of baseline gonadal steroid production as adults (21,22) and suppressed ovarian function (23,24); low energy intake may also affect lactational performance in breast-feeding women (25,26). Women have longer periods of lactational amenorrhea after childbirth under conditions of low caloric intake even when the intensity and duration of lactation are controlled (27,28).
This is attributed to the higher relative metabolic load of lactation. Fecundity rates are relatively low with babies born at 4–5-year intervals (29), although menstrual cycles are generally preserved. These data again suggest that CR is associated with ovulation disturbances. Low energy intake has also been linked with a lower basal metabolic rate in some but not other studies and is probably dependent on protocol and method (30).

Additionally, there is evidence of immune suppression in populations subject to chronic low-energy intakes (31,32). Cell-mediated immunity appears to be more affected than humoral immunity (33). Caloric supplementation of undernourished populations has been observed to lead to reductions in gastrointestinal and upper respiratory infections (34).

Many populations are also subject to recurring acute reductions in energy intake associated with seasonal reductions in food availability. This is particularly true for populations relying on subsistence agricultural activities. These seasonal food shortages have been associated with increases in morbidity and mortality (particularly among children), decreases in levels of ovarian function in women, and decreases in conception rates (35–40). However, it should be noted that seasonal food shortages often coincide with a rainy season, when disease transmission rates are high.

The effects of chronic low-energy intake on old-age morbidity has not been a focus for most of these studies. However, a study by Kagawa (41) reported on the diet and life span of Japanese living in different areas of their country. The Japanese government has kept records of birth and death since 1892 and conducts annual diet surveys, thus providing a wealth of information on aging and nutrition. Kagawa reported that, compared to mainland Japan, inhabitants of the island of Okinawa consume an average of 20% fewer calories while at the same time they consume adequate intakes of protein and micronutrients. This reduction in energy intake is associated with a 2- to 40-fold increase in the incidence of centenarians, implying an association between voluntary CR and life span. While these data are consistent with the hypothesis that CR increases life span in humans, it is possible that there are other unmeasured (e.g., genetic) differences between Okinawa and mainland Japan that might account for the findings on centenarians.

Other studies of traditional forager populations have documented high adult mortality associated with consumption of diets low in energy but apparently sufficient in protein and other nutrients (29,42,43). However, the concomitant lack of medical care and primitive sanitary conditions undoubtedly play an important role.

Restrained eating might be speculated to be another form of CR. The phenomenon of “cognitive dietary restraint” is a form of psychological awareness of food and weight such that individuals believe they must restrict energy intake to prevent becoming overweight. However, it has been controversial whether or not restrained eaters consume less dietary energy than unrestrained eaters (44–46), and it is thus uncertain whether dietary restraint is a good model for CR in humans or whether it should be viewed primarily as a psychological trait.

In unpublished studies by S.B. Roberts and colleagues, an attempt was made to identify otherwise well-nourished women who practice voluntary CR for the purpose of preventing weight gain. Women classified as “restrained eaters” (47,48) appeared to be a good potential group to study based on a previous study documenting low levels of energy expenditure (49). Table 1 summarizes some of the findings to date. As can be seen, there was no significant difference between restrained and unrestrained eaters in any of the key parameters measured. In particular, no differences were observed in total energy expenditure, resting metabolic rate, or body temperature, three variables that have been reproducibly affected by CR in animal models. On the other hand, restrained eaters did significantly underreport energy intake relative to total energy expenditure (reflective of usual energy needs), suggesting that previous reports of low energy intake in restrained eaters may have been inaccurate. These results suggest that restrained eaters are not voluntarily restricting their energy intake into a range where equivalent adaptations to those seen in CR animal models occur, and are thus not a good model for human CR.

It should also be noted that all published studies of restrained eating that have observed menstrual cycle and hormonal differences compared with nonrestrained women have shown that ovulatory disturbances occur at a higher prevalence (44–46). In addition, recent data show high levels of 24-hour urinary free cortisol excretion in women scoring high on the restraint scale compared with those scoring low (50). At the very least, these data—combined with the other studies described above—suggest that voluntary CR may be difficult in humans, and raise the question of whether the degree of CR found to induce maximum beneficial effects in animals is a realistic possibility. Only intervention studies of controlled CR will address this important issue.

Effects on Bone
Epidemiological studies have shown that women with a low body mass index (BMI) and reporting a history of weight loss have lower bone mass and greater fracture risk than heavier women (51–54) and men (55,56). For example, a 3-year study of 827 older women showed that thinner women with larger weight loss had considerably less bone

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<th>Table 1. A Summary of Some Characteristics of 58 Restrained and Unrestrained Eaters*</th>
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<tr>
<td><strong>Unrestrained</strong></td>
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<tr>
<td><strong>Age (years)</strong></td>
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<td><strong>BMI (kg/m²)</strong></td>
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<td><strong>Body fat (% weight)</strong></td>
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<td><strong>Total energy expenditure (MJ/d)</strong></td>
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<td><strong>RMR (MJ/d)</strong></td>
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<td><strong>Body temperature (F)</strong></td>
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<td><strong>Fasting serum cholesterol (mg/dl)</strong></td>
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<td><strong>BMD (g/cm²)</strong></td>
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*Values are means ± SD. BMI, body mass index; RMR, resting metabolic rate; BMD, bone mineral density. Source: Unpublished data of S.B. Roberts.
at the femoral neck than the average, and 3–5 times more than subjects whose weight had increased (54). However, it is likely that there are differences between the effects of intentional and unintentional weight loss that cannot be addressed in observational studies (52). For example, the bone loss associated with unintentional weight loss could be secondary or a companion to other factors such as disease state. Preliminary data from a population-based cross-sectional study of 1,065 men and women aged 25–96 years from British Columbia’s cohort of the Canadian Multicentre Osteoporosis Study show that reported voluntary weight cycling behavior (loss and gain of 10 pounds) is significantly and negatively related to BMD (in regression equations that control for weight, height, calcium intake, energy expenditure, and gender). In addition, with increasing number of weight cycling episodes there is increased likelihood of reporting prevalent fragility fractures (J.C. Prior, personal communication). It should also be noted that retrospective studies cannot clearly differentiate between the effects of negative energy balance and specific nutrient deficiencies (i.e., calcium and vitamin D intake) on the parameters measured.

**INTERVENTION STUDIES OF CR IN HUMAN POPULATIONS**

**Effects on Metabolic Rate and Metabolic Variables**

There are many research publications on the effects of weight loss on metabolic rate and metabolic variables. In the early studies by Keys and colleagues (57), energy intakes of approximately 1500 kcal per day (i.e., to about 43% of weight maintenance energy intake) were used to induce weight loss over periods lasting up to 6 months so that physiological effects could be noted. Changes in many variables were observed, including decreased metabolic rate, decreased pulse, and decreased blood pressure. All observed metabolic effects were consistent with the effects of CR seen in animal models. Adverse psychological effects, including decreased vitality, were also noted, but (as with the natural CR experiments) the diets consumed by the volunteers were not designed as optimal CR regimens.

Concerning the data from studies of commercial weight-loss diets, there is considerable information on their metabolic effects, but the extremely low energy contents (58) make them unlikely models for long-term CR in humans. Short-term CR studies that relate study variables to energy insufficiency (59,60) are also inappropriate models for human CR because, like weight loss studies, the time interval over which changes are assessed is limited. Nevertheless, they do typically show changes in metabolic variables consistent with the results of CR studies in animals.

The study of Leibel and associates (61) monitored changes in metabolic rate and key energy metabolism hormones in response to a 10% loss of body weight and a subsequent 1–3-month period of weight stabilization in normal-weight men and women (BMI of ~25, <40 years of age). These results were consistent with other related experiments (62,63). Results from that study are summarized in Table 2.

The weight-prevention intervention study of Simkin-Silverman and colleagues (64), in which a weight-stable diet and exercise group was compared to a control group who gained weight, noted improvements in the intervention group in insulin sensitivity, total cholesterol, HDL, and triglycerides over 5 years. However, it should be noted that the relative effects of exercise and CR cannot be distinguished in this intervention.

To our knowledge, there is only one study—Vallejo (65)—that can be considered directly relevant to the question of long-term CR in humans. Vallejo reported on a controlled investigation of the effects of CR on morbidity and mortality in a group of 120 retired members of religious institutions, the stated purpose of which was to see whether early studies by McCay and Cromwell (66) might be relevant to humans. Over a 3-year period, 60 control subjects received a diet containing 2300 kcal/day. The 60 experimental subjects received 2300 kcal every second day and on the other days received 1 liter of milk and 500 g fruit, giving an overall average energy intake in the experimental group of approximately 1500 kcal/day (thus a restriction to approximately 65% of the controls). In a reanalysis of the data from this study, Stunkard (67) determined that the lower energy intake in the restricted group was associated with a significantly lower rate of admission to the infirmary (123 days compared to 219 days, p < .001) and a nonsignificant lowering of death rate (6 experimental subjects, compared to 13 controls). Despite the apparent importance of these findings, caution has to be used when interpreting the results because of the extreme brevity of the original report (2 pages) and the complete lack of information on the gender breakdown in the two groups, the diet composition, and other experimental details that might have influenced the observed differences between the groups.

**Effects on Bone**

Clinical studies show that weight loss in obese women increases the rate of bone turnover (68) and bone loss (69–74), and the response may differ during acutely low energy intake and fasting (75,76). The mechanism for these changes is not known but may possibly be related to a slow rise in parathyroid hormone (PTH), and a reduction in sex hormones (69,77). In addition, cross-sectional data in women reporting a history of weight cycling show lower BMD, even when matched for current weight (77). Concerning the

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<th>Table 2. Measure at Weight–10% Relative to Measurements Determined at Usual Body Weight*</th>
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<tr>
<td>↓TEE (~348 ± 87 kcal/day (~15%))</td>
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<tr>
<td>↓RMR (~101 ± 41 kcal/day (~8%))</td>
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<tr>
<td>↓NRMR (~234 ± 85 kcal/day (~36%))</td>
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<td>↓RQ during unloaded exercise (~—9%)</td>
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<tr>
<td>↓Skeletal muscle efficiency during unloaded exercise (~13%)</td>
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<td>↓Muscle glycolytic/aerobic-oxidative enzyme ratio (~10%)</td>
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<td>↓Expression of UCP3 in skeletal muscle (~45%)</td>
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<td>↓Urinary catecholamine release and sympathetic nervous system tone</td>
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<td>↓Parasympathetic nervous system tone</td>
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*TEE, total energy expenditure; RMR, resting metabolic rate; NRMR, non-resting metabolic rate (TEE–RMR); RQ, respiratory quotient; UCP3, uncoupling protein 3.

Source: Data of R. Leibel (49) and unpublished.
magnitude of the effect of weight loss on BMD, a 10% reduction in body weight has been shown to result in a ~1–2% loss in BMD (69,71–73). There is no information on how the rate of weight loss affects bone turnover and loss.

The relationship between bone and other components of body composition during weight loss is not clear, especially with respect to specific sites (i.e., primarily cortical compared to trabecular sites). In one relatively short-term study (n = 45), postmenopausal women were examined for 1 year (78). Although the study design did not include weight loss, a subset of the women lost weight over the course of the study. It was found that fat-free mass and weight were associated with regional BMD after adjustment for initial bone mineral values (78).

**RECOMMENDATIONS FOR RESEARCH**

Currently there is no published information on the effects of CR on life span in nonhuman primates. Such information will ultimately become available from the ongoing nonhuman primate studies and will be an important component of the information needed to judge the potential effects of CR in humans. In addition, specific studies are needed in humans because of the question of feasibility in humans and also the potential for CR to impact lifestyle and behavior in unacceptable ways not detected in controlled nonhuman primate studies. The following recommendations relate specifically to studies in humans.

**Intervention Studies**

Long-term caloric restriction intervention studies need to be performed, both as studies decreasing energy intake and studies preventing weight gain. Ideally these studies would be randomized, but blinding would not be possible.

**Specific surrogate endpoints.—**Specific surrogate endpoints to assess the positive and negative effects of CR should be chosen from candidates identified in animal models of CR. In addition, the following endpoints should be considered as potential variables affected by CR in humans.

**Energy metabolism and body composition.—**Measurements of energy expenditure will be an essential component of protocol validation (i.e., determining that subjects are truly restricted in calories or more physical activity).

- In particular, measurements of total energy expenditure are needed to determine the extent of total caloric restriction, and measurements of resting metabolic rate are needed to assess the extent of caloric restriction after other volitional compensatory changes (e.g., reduced physical activity).
- Body fat and fat-free mass are primarily outcome variables in any CR trial. In addition, the quality of lean tissue should be measured, as assessed by functional measures of strength, work performance, and maximum oxygen consumption.
- Body temperature should be measured using appropriately sensitive and accurate techniques, for example, radiotelemetry as in the nonhuman primate studies. Although somewhat variable between species, body temperature is usually reduced in animal models of CR and tends to be one of the most stable and easily measured variables that can help determine CR status. Studies should include assessment over 24-hour periods and, in women, during different phases of the menstrual cycle.

**Endocrine responses.—**Hormonal responsiveness to CR will also be a critical indicator of the long-term effects of CR.

- Hypothalamic-pituitary reproductive function and hormone levels and binding, including rates of nonovulation, luteal phase lengths, and effects on fertility in men as well as in women, and reproductive function.
- Hypothalamic-pituitary-adrenal axis including cortisol excretion.
- Leptin and insulin levels and function.
- Thyroid hormone dynamics including binding and active forms.

**Nutrient utilization.**

- Measurement of insulin sensitivity and circulating glucose.
- Protein metabolism and turnover.

**Cardiovascular and respiratory measures.**

- Blood pressure.
- Cardiac function.
- Pulmonary function.

**Bone metabolism.**

- Measurements of bone mass at different sites using DXA, or other accurate methods as they become available.
- Bone markers of resorption and formation.
- Quantitative histomorphometry.
- Bone-regulating hormones (parathyroid hormone, calcitonin, vitamin D, estrogens, progesterone, etc.) and/or cytokines (e.g., IL-1, IL-6).
- Calcium metabolism measures (i.e., balance, adsorption).
- Fracture risk (prospectively documented including degrees of trauma in clinical trials and studies of bone quality and strength in animal models).

**Immune function.—**Immune function will be an important outcome variable, with measurements including cell-mediated and humoral immune function, cytokines, and acute phase proteins, and propensity to infection as measured by logged disease incidence.

**Quality of life.—**Measures could include psychosocial function (including cognitive function and memory), sleep, and self-reported vitality. These parameters are important because they will provide important information relevant to the application of CR in free-living humans.

**Observational Studies**

Observational studies on long-living lean individuals who may have restricted their caloric intake or had it restricted by natural events may potentially be valuable sources of information on human CR, if truly CR popula-
tions can be identified that are consuming (and have previously consumed) an otherwise healthy diet. After successful identification of populations undergoing voluntary CR (based on energy metabolism parameters), it would be interesting to evaluate the parameters described above under intervention studies, including body composition, endocrine, cardiovascular, metabolic, bone, and psychosocial function.

Potentially suitable populations might include (a) lean individuals who have survived to old age, and (b) any available populations who report having limited their weight in one of three ways: energy intake decrease, energy expenditure increase, or both.

**Recommendations on Methodologic Issues**

**Age Groups for All Types of Studies**

Different age groups are likely to respond differently to energy restriction. Young adults would seem to be an ideal, initial study group to be followed over time. It should also be noted that although it may be easier to conduct CR studies in elderly adults, because of their reduced control of food intake, studies in animals have not observed anti-aging effects of CR when started late in life. In normally menstruating young women, the usual increase in energy intake during the luteal phase of the menstrual cycle may make a uniform pattern of CR across the menstrual cycle more difficult than an energy intake that takes hormonal patterns into account.

**Ethnic and Gender Studies**

It cannot be assumed that men and women or people from different ethnic/racial groups will respond to CR in a similar manner, and account of this possibility should be taken in study designs.

**Observational Studies**

Validating interventional and observational studies to ensure that subjects are truly CR is a critical component of any human CR study. The doubly labeled water method can help provide such a validation for overall energy intake, together with measurements of energy intake and body temperature. Resting metabolic rate may also provide an index of the extent to which reduced energy intake is having measurable metabolic effects, and activity logs can also help document the effects of changes in energy intake.

**Long-term Intervention Studies**

(a) What is the minimum duration that can provide information relevant to the effects of long-term CR in humans? Although only extremely long-term studies (1+ decades) will provide mortality data and data on some types of morbidity, what surrogate endpoints can potentially provide an interim index of the effects of CR in shorter term studies? A minimum duration of two years of CR after the weight-loss period of the intervention has been completed is suggested as appropriate for surrogate measures. Note that animal CR studies (including on primates) are essential for making the final definition of surrogate measures, together with aspects of human metabolism and psychology that would provide absolute endpoints in their own right (e.g., cognitive function).

(b) What level of energy intake is it appropriate to aim for in human CR intervention studies? The early work of Keys and colleagues suggests that energy intakes of approximately 1500 kcal/day in healthy men (equivalent to approximately 40% of normal intake) produce unsustainable symptoms for voluntary CR, including loss of vitality and perceived energy level, and persistent weight loss over 6 months. This suggests that CR to 40% of usual intake is too extreme in humans. On the other hand, insufficient CR might fail to mimic signs of CR in animals even if such signs might be apparent at lower levels of CR. Therefore, initial studies should focus on the most moderate levels of CR that, based on animal studies, should produce evidence of beneficial effects if such effects are to be seen. Because it can be hard to measure energy intake on a routine basis, an expected range for weight reduction can be developed and used as a criterion for human CR studies. Given that energy expenditure is thought to decrease disproportionately to the decrease in energy intake, an effective initial target range for weight reduction in CR interventions may tentatively be set at 15–20% of initial body weight starting from the nonobese state. Ultimately, several CR studies at different levels of dietary restriction will be needed to assess the potential benefits and disadvantages of CR in human populations.

(c) Types of CR diets. The types of diets provided during CR also require careful consideration. At a minimum, all diets should provide at least the RDAs/DRIs for essential nutrients when consumed at the anticipated level of energy intake. Monitoring of potential nutrient deficiencies during CR should be considered. In addition, some diets may be easier to consume at low levels of energy intake than others (e.g., diets with low energy density), but would providing a diet that is easy to underconsume reduce the potential effectiveness of CR? It is possible that only diets that cause hunger may activate energy-conserving mechanisms and have long-term benefits associated with CR in animal models, and this possibility needs to be explored by parallel evaluation of different dietary regimens.

(d) Compliance assessment. Compliance assessment will be a critical factor of any human CR intervention study, because subjects will have to substantially change their dietary patterns and some may become noncompliant. Measurements of total energy expenditure using the doubly labeled water method will help determine whether average energy intake matches that which is prescribed. In addition, providing food during the weight-loss period of the study will maximize weight loss success and compliance.

(e) Studies of body composition. In studies of body composition, the focus of emphasis is primarily on fat, muscle, and bone.

**Recommendations for Resources and Infrastructure**

Doubly labeled water is the best method for determining energy expenditure in free-living individuals and, thus, energy intake. It is also an excellent tool to measure compliance during intervention studies and to assess whether free-living populations are truly practicing CR. This method is difficult and expensive, and now has the additional disadvantage that one of the isotopes (18O) is currently unavail-
able. Mechanisms to ensure a reliable supply of isotope and to improve the cost and ease of doing such studies need to be worked out. In addition, alternative methods that predict total energy expenditure accurately need to be developed.

General clinical research centers and centers for clinical nutrition are necessary for the carrying out of metabolic studies in human volunteers and for providing food during the initial phase of human CR.

Clinical trials of human CR are necessary and will require appropriately trained personnel and adequate resources in order to be carried out effectively. These trials are especially laborious with respect to provision of food (recommended to promote compliance at least during weight loss), behavioral monitoring, and general patient management.

There are methodological problems relating to bone measurements by DXA that need resolution before the method is used widely for studies of CR in humans (81,82). Specifically, the strong correlation between change in BMD and both weight and weight change raises an issue of whether observed results may be confounded by technical problems in measurement (53). One study suggested that there is a significant reduction in instrument sensitivity in studies attempting to measure changes in bone with weight loss (83). Unfortunately, that study (n = 14) did not include a control group that did not lose weight. Others have attempted to control for the overlying fat tissue (53,70), and believe it did not present problems in their bone measurements after weight loss. Artifactual and real changes in bone mineral content, bone area, and BMD need to be addressed in future studies using DXA to assess bone changes after weight loss. There are also methodological problems related to fatness versus muscle, and appropriate methods for maximizing measurement sensitivity should be employed. For example, standardized methods for measuring DXA in the obese individual should be established (i.e., slow scan mode if necessary). These studies should be population-specific and control for initial body weight and different amounts of weight loss.

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Because part of the charge to the panel was to provide the National Institute on Aging (NIA) with independent recommendations for research, Dr. Lane, as an NIA staff member, did not participate in the development of the Recommendations for Research section of this report.

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