

Calorie restriction and aging: review of the literature and implications for studies in humans¹⁻³

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ABSTRACT Calorie restriction (CR) extends life span and retards age-related chronic diseases in a variety of species, including rats, mice, fish, flies, worms, and yeast. The mechanism or mechanisms through which this occurs are unclear. CR reduces metabolic rate and oxidative stress, improves insulin sensitivity, and alters neuroendocrine and sympathetic nervous system function in animals. Whether prolonged CR increases life span (or improves biomarkers of aging) in humans is unknown. In experiments of nature, humans have been subjected to periods of nonvolitional partial starvation. However, the diets in almost all of these cases have been of poor quality. The absence of adequate information on the effects of good-quality, calorie-restricted diets in nonobese humans reflects the difficulties involved in conducting long-term studies in an environment so conducive to overfeeding. Such studies in free-living persons also raise ethical and methodologic issues. Future studies in nonobese humans should focus on the effects of prolonged CR on metabolic rate, on neuroendocrine adaptations, on diverse biomarkers of aging, and on predictors of chronic age-related diseases. *Am J Clin Nutr* 2003;78:361-9.

KEY WORDS Dietary restriction, energy expenditure, aging, life span, nonobese persons, oxidative stress, metabolic rate, insulin sensitivity, neuroendocrine axis

INTRODUCTION

Evidence that calorie restriction (CR) retards aging and extends median and maximal life span was first presented in the 1930s by McCay et al (1). Since then, similar observations have been made in a variety of species including rats, mice, fish, flies, worms, and yeast (2, 3). Although not yet definitive, results from the ongoing calorie-restriction studies in monkeys also suggest that the mortality rate in calorie-restricted animals will be lower than that in control subjects (4-7). Furthermore, calorie-restricted monkeys have lower body temperatures and insulin concentrations than do control monkeys (4), and both of those variables are biomarkers for longevity in rodents. Calorie-restricted monkeys also have higher concentrations of dehydroepiandrosterone sulfate (4). The importance of dehydroepiandrosterone sulfate is not yet known, but it is suspected to be a marker of longevity in humans (8, 9), although this is not observed consistently (10). In humans, a major goal of research into aging has been the discovery of ways to reduce morbidity and delay mortality in the elderly (11, 12). The absence of adequate information on the effects of CR in humans reflects the difficulties involved in conducting long-term calorie-restriction studies, including ethical and methodologic considerations.

Naturally occurring episodes of CR in human populations are not uncommon in some parts of the world. However, it is important to note that most of these populations are exposed to energy-restricted diets that are lacking in protein and micronutrients. CR in these populations is often associated with short stature, late reproductive maturation (13), lower baseline gonadal steroid production in adults (14, 15), suppressed ovarian function (16), impaired lactation performance (17), impaired fecundity (18), and impaired immune function (19, 20). The pioneering studies by Keys et al (21) found that severe CR induced changes in many variables, including metabolic rate, pulse, body temperature, and blood pressure. However, that diet also was of poor quality and induced many adverse psychological effects.

A few observational studies reported the effects of prolonged CR, in the context of high-quality diets, on health and longevity. Kagawa (22) carefully analyzed data documenting the prevalence of centenarians on the island of Okinawa (Japan). Total energy consumed by schoolchildren on Okinawa was only 62% of the "recommended intake" for Japan as a whole. For adults, total protein and lipid intakes were about the same, but energy intake was 20% less than the Japanese national average. The rates of death due to cerebral vascular disease, malignancy, and heart disease on Okinawa were only 59%, 69%, and 59%, respectively, of those for the rest of Japan. Whereas these data are consistent with the hypothesis that CR increases life span in humans, there probably are other, unmeasured differences between Okinawa and mainland Japan, including genetic or other environmental factors. However, Okinawans who move away from the island (and presumably abandon their protective lifestyle patterns) have mortality rates higher than those in Okinawans who remain on the island (23). To our knowledge, only one study investigated the effects of long-term CR (with a diet of reasonable quality) on health and longevity in nonobese humans and a control group (24). This study was conducted in 120 men, of whom 60 were randomly assigned to the control group and 60 to the calorie-restricted group. The control group was fed ≈ 9600 kJ/d. Calorie-restricted subjects received 1 L milk and 500 g fruit every other day, which

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TABLE 1
The effects of chronic calorie restriction on markers of aging in rodents and nonhuman primates and predicted outcomes in nonobese humans¹

| | Rodents | Nonhuman primates | Expected outcomes in nonobese humans |
|----------------------------|------------------|-------------------|--------------------------------------|
| DHEAS | ? ² | ↑ | ↑ |
| Insulin | ↓ | ↓ | ↓ |
| Body temperature | ↓ | ↓ | ↓ |
| Energy expenditure | | | |
| Total ³ | ↔ ⁴ ↓ | ↔↓ | ↓ |
| Sedentary ³ | ↓ | ↓ | ↓ |
| Voluntary activity | ↑ | ↔ | ↓ |
| Oxidative stress | ↓ | ↓ | ↓ |
| Fat mass and fat-free mass | ↓ | ↓ | ↓ |
| Visceral fat | ↓ | ↓ | ↓ |
| Intramyocellular lipids | ? | ? | ↓ |
| Insulin sensitivity | ↑ | ↑ | ↑ |
| IGF-1 | ↓ | ↓ | ↓ |
| Thyroid axis | ↓ | ↓ | ↓ |
| HPA axis | ↑ | ? | ↓ |
| Gonadotropic axis | ↓ | ↔ | ↓ |
| Somatotropic axis | ↓ | ? | ↓ |
| Sympathetic activity | ↓ | ? | ↓ |

¹Measured effects of calorie restriction on the above markers of aging were obtained from references 32–43 for rodents and 4 and 44–50 for monkeys. DHEAS, dehydroepiandrosterone sulfate; IGF-I, insulin-like growth factor-I; HPA, hypothalamus-pituitary-adrenal.

²Effect not known.

³Adjusted for fat-free mass.

⁴No effect.

led to an overall mean energy intake of ≈6300 kJ/d (or a 35% restriction from the intake of the control group). This regimen was implemented for 3 y. Stunkard (25) reanalyzed these data and reported less time in the infirmary (123 compared with 219 d) and a nonsignificant difference in the death rate (6 compared with 13 deaths) in calorie-restricted subjects than in control subjects, respectively, which suggests that chronic CR may prolong life span in humans.

Results from the Biosphere 2 experiment also give us an insight into the effects of long term CR in nonobese humans. Biosphere 2 is a 12 750-m² (3.15-acre), enclosed, glass and steel structure that was constructed as a self-contained ecologic “miniworld” and prototype planetary habitat. From 26 September 1991 to 26 September 1993, 8 healthy subjects (4 men, 4 women) lived inside Biosphere 2, during which time the enclosure was materially sealed, ie, no material passed in or out, except small items used for research purposes. Unexpectedly, the amount of food grown inside was less than originally predicted, and, as a result, all 8 biospherians experience a marked weight loss (26). Numerous changes in physiologic, hematologic, hormonal, and biochemical variables were observed (27). These changes mimic many of the effects previously observed in rodent calorie-restriction studies, but they cannot be equated with increased longevity in nonobese humans.

It is also not known whether creating a relative energy deficit by increasing physical activity (PA) without modifying other health behaviors will increase life span. Increased activity (to achieve a relative energy deficit of 30%) did not extend maximal life span in rodents, although average life span was increased (28).

Increased activity was also associated with some traits of CR, including improved insulin sensitivity, decreased fat mass (FM), and reduced tumor incidence. Holloszy (29) observed that adding PA to CR did not interfere with the life-extension effects of CR. Currently, no data exist on whether PA (or a combination of CR and PA) will extend life span in humans. Observational studies suggest that PA improves the quality of life and prevents the onset of chronic disease (30, 31). Increasing PA to create a relative energy deficit may improve compliance and may be associated with a lower rate of attrition in humans.

Many of the effects of prolonged CR have been characterized (Table 1), but the mechanism or mechanisms by which CR increases life span are still debated. An initial hypothesis was that delayed sexual maturation might be one of those mechanisms through which CR exerts its effect on longevity, until it was shown that CR initiated in older animals also extends life span (51, 52). Reduced metabolic rate is another possible explanation for the anti-aging effects of CR, with the consequent reduction in free radicals underpinning this observation. However, many other metabolic alterations are associated with CR. For instance, short-term CR in humans and long-term CR in animals alter insulin sensitivity, the secretion of many hormones, and sympathetic nervous system activity. CR also alters the gene expression profile in muscle, heart, and brain. Any (or a combination) of these biological changes may retard aging, and evidence for each of these theories will be presented in this review.

CALORIE RESTRICTION AND ENERGY METABOLISM

CR is hypothesized to lessen oxidative damage by reducing energy flux and metabolism, or the “rate of living,” thereby influencing the aging process (53, 54). We know that CR results in a loss of weight and tissues and a reduction in the rate of metabolism. A portion of this decline is the result of reduced energy intake and the consequent decrease in the thermic effect of food, whereas another portion is due to the reduced size of the metabolizing mass. However, whether there is also a “metabolic adaptation,” defined here as a reduction in the metabolic rate that is out of proportion to the decreased size of the respiring mass, is a subject of continued debate. In their investigation of the biology of semistarvation, Keys et al (21) defined metabolic adaptation as “a useful adjustment to altered circumstances.” More recently, a 1985 FAO/WHO/UNU report proposed a definition of adaptation as “a process by which a new or different steady state is reached in response to a change or difference in the intake of food or nutrients” (55). In this context, the adaptation can be genetic, metabolic, social, or behavioral. The important question is whether CR reduces energy expenditure (EE) more than would be expected to result from the changes observed in FM and fat-free mass (FFM).

McCarter et al studied basal metabolic rate in rats after restricting their energy intake for 6 mo (56) and for their entire life span (57). Rats fed ad libitum and rats on a food-restricted regimen (40% CR) had similar metabolic rates, as measured per kilogram of FFM. However, these data were criticized by Lynn and Wallwork (58), who suggested that oxygen consumption should be adjusted for functional metabolic mass instead of for FFM. Furthermore, it is now well accepted that dividing EE by body size leads to a mathematical artifact because of the fact that the regression line between EE and body size does not go through the zero intercept (44, 59, 60). In contrast with the findings of McCarter et al, Ballor (32) observed a decrease in 23-h resting oxygen uptake

after 11 wk of moderate (25%) and severe (50%) CR. However, after correction was made for body weight, only severe CR lowered the metabolic rate, even when metabolic rate was expressed per kg^{0.75}. After 6 wk of severe (40%) CR, the mean metabolic rate was 14% lower after adjustment for body size (45). Taken together, these results indicate that the absolute metabolic rate declines after CR, but this reduction may be only transient after adjustment for changes in body weight and body composition (53). However, before a final conclusion can be drawn, most of these data should be reevaluated by using an appropriate method of normalizing metabolic rate for body size and composition (44). Recently, when reanalyzing the data, Blanc et al (59) clearly showed that, in most instances and in all species, CR causes a decrease in resting EE. In fact, monkeys subjected to 11 y of CR had a reduced total EE (TEE) that was attributable to a 250 kJ/d reduction in resting EE, independent of reduced FFM (59). Other studies in monkeys showed that 30 mo of CR reduces nighttime and 24-h EE (61). Furthermore, 10 y of CR in monkeys also resulted in sustained reductions in TEE by doubly labeled water methods, even after correction for FFM (41).

The first experiments on the effect of energy restriction in humans were performed in lean men by Keys et al (21) in the 1950s. In these classic experiments, lean volunteers received 50% of their habitual intake for 24 wk. Basal metabolic rate was decreased after adjustment for body surface area (−31%), body weight (−20%), and cell mass (−16%). The reduced metabolic rate was paralleled by a reduction in temperature that indicated a real metabolic adaptation in these lean subjects (63). Most other studies investigating the effects of energy restriction on energy metabolism were performed in the obese. In several of these studies, a very-low-energy diet resulted in a decrease in basal metabolic rate that was still significant after adjustment for differences in body weight, FFM, or both (64–66). A meta-analysis of studies in formerly obese persons found a lower resting metabolic rate, even after adjustment for body size and body composition (67). Careful studies in formerly obese persons showed that energy turnover was ≈15% less than that in never-obese persons of the same body composition (68–71). In lean subjects, maintenance of body weight 10% below initial weight also reduced EE by 10–15%, even after adjustment for FFM (68). These results were confirmed by a direct measure of TEE using a respiratory chamber and the doubly labeled water method (72). Part of this adaptation may be related to the cost of PA, as elegantly shown by Weigle and Brunzell (71). It is relevant that we clearly identified a metabolic adaptation in the 5 persons from Biosphere 2 who agreed to participate in follow-up measures of energy metabolism after almost 2 y of CR (26). The subjects, measured within a week after their exit from Biosphere 2, had decreases in adjusted 24-h EE and spontaneous PA in a respiratory chamber when compared with 152 control subjects. However, within the confinement of Biosphere 2, TEE measured by doubly labeled water was not characteristically low. This was probably due to the relatively high level of PA required to keep Biosphere 2 in operation and to harvest food inside the enclosure.

In summary, there is evidence that a metabolic adaptation develops in response to CR and loss of weight in humans. The reason for the apparently paradoxical difference between rodents and humans with regard to an adaptation in EE in response to CR may be related to the erroneous way in which physiologists express rodent energy metabolism data (60) or to differences in metabolism between rodents and humans. Other possible reasons are that the methods for measuring human EE are more sensitive

than are those for measuring rodent EE, and investigators can obtain the full cooperation of the subjects.

CALORIE RESTRICTION AND BODY COMPOSITION

CR prevents the increases in visceral FM and intramyocellular lipid deposition that are generally observed with aging (73, 74). In rodents, however, no association has been observed between FM and longevity in animals fed ad libitum, and, in fact, a positive correlation was observed between FM and longevity in calorie-restricted animals (75). This finding led to the conclusion that changes in FM brought about by long-term CR do not influence longevity.

In the past 5–10 y, much evidence of the importance of adipose tissue has appeared. Adipocytes secrete numerous cytokines that can affect substrate oxidation, EE, insulin sensitivity, and the neuroendocrine system (33). Furthermore, short-term CR in obese humans, independent of changes in FM, alters the expression of numerous adipocytokines (33), and this change is associated with an improvement in markers for age-related diseases such as atherosclerosis and type 2 diabetes. Whether alterations in body composition brought about by CR positively influence markers of longevity in nonobese subjects has not been investigated. Recently, Gabrieli et al (76) observed that the surgical removal of visceral adipose tissue restored peripheral and hepatic insulin sensitivity in aging Zucker rats. We know that visceral fat is reduced by CR (77) and is related to improvements in insulin sensitivity in the obese. However, it is still debated whether the accumulation of visceral fat is the major cause of insulin resistance (78). Intramyocellular lipid concentrations are also related to insulin resistance in lean individuals (79). Intramyocellular triacylglycerol content is higher in obese persons than in nonobese persons, and it is reduced by weight loss (80, 81). Whether the improvements in insulin sensitivity in response to CR in nonobese persons are caused by a reduction in intramyocellular lipids is unresolved.

CALORIE RESTRICTION AND OXIDATIVE STRESS

The oxidative stress hypothesis of aging is supported by a number of observations: 1) life span is inversely correlated with metabolic rate in a wide variety of animals, and it is directly related to the amount of reactive oxygen species (ROS) produced (82); 2) overexpression of antioxidative enzymes or activation of defensive mechanisms against oxidative stress retards aging and extends life span in some organisms (83, 84); and 3) CR reduces oxidative stress in various species, including mammals (3, 34, 85).

Normal energy metabolism in aerobic organisms is coupled to the generation of ROS. In fact, 2–5% of oxygen consumption is not associated with the oxidative metabolism of fuels but is associated with the production of highly reactive oxygen molecules such as the superoxide radical (O₂^{•−}), hydrogen peroxide, and the hydroxyl radical (OH[•]). Therefore, reducing metabolic rate by using CR may reduce oxygen consumption, which could decrease ROS formation and potentially increase life span.

Steady state measures of oxidative damage represent equilibrium among the rate of ROS generation, the rate of oxygen scavenging, and the rate of repair. Aging may therefore be retarded not only by a decrease in the production of ROS, but also by an increase in the removal of ROS by the mechanisms described above. For example, a transgenic fly that overexpresses both



superoxide dismutase and catalase has a 30% extension of life span associated with a lower amount of protein oxidative damage and a delayed loss of physical performance (83, 86). Similar results were obtained in *Caenorhabditis elegans* with low-molecular-weight synthetic superoxide dismutase and catalase mimetics (84). However, there is so far no report in mammals of the extension of life span in transgenic mice that overexpress catalase or superoxide dismutase. The p66^{shc} knockout mouse has an extended life span caused by a gene deletion that may be directly related to the cellular response to oxidative stress (87). Superoxide dismutase–heterozygous knockout mice on the other hand, have greater amounts of DNA damage [evidenced by elevated 8-oxoguanine (8-oxoG)], but median and maximal life spans did not differ from those of control mice (A Richardson, personal communication, 2002).

Currently, no standard measures for assessing oxidative damage are established. One method of determining the amount of protein oxidation induced by ROS is to measure carbonyl groups in serum (88). In obese humans, protein carbonylation has been significantly associated with age and was reduced after 4 wk of CR (89). ROS also increases the amount of lipid peroxidation. Isoprostanes are prostaglandin-like products of arachidonic acid peroxidation that circulate in plasma and are excreted in urine (90). Urinary isoprostanes (8-*iso*-prostaglandin F_{2α}) are higher in smokers (90, 91), in persons who consume alcohol (92), and in ischemia-reperfusion syndromes (93), Alzheimer disease (94), and chronic obstructive pulmonary disease (95), and there is some evidence that isoprostanes are higher with aging (96). There is also evidence that Okinawan centenarians have lower rates of lipid peroxidation than do Okinawan septuagenarians, which indicates less free radical attack [Internet: <http://okinawaprogram.com/study.html> (accessed 17 January 2003)]. Furthermore, urinary isoprostanes were increased in obese women compared with control subjects and were significantly reduced by weight loss (97).

Much attention has been paid to the effects of ROS on DNA damage. ROS can induce the formation of several base adducts in DNA, which are implicated in mutagenesis, carcinogenesis, and neurologic disorders (98). Of major interest is the fact that the amount of DNA damage correlates with the metabolic rate in various animals, which suggests that ROS generated by aerobic energy metabolism may be a major cause of spontaneous DNA damage (99). An abundant marker of DNA damage by free radical attack is 8-oxoG (100). Free radical attack on DNA can also give rise to baseless (apurinic/apyrimidinic) sites. Moreover, the repair of 8-oxoG can give rise to apurinic/apyrimidinic sites as an intermediate in their repair. The presence of apurinic/apyrimidinic sites in DNA can cause mutations (101, 102) or be lethal to the cell (101). Notably, the formation of both 8-oxoG and apurinic/apyrimidinic sites increases with age (103, 104), and a close association between oxidative DNA damage (assessed by the urinary excretion of 8-oxoG) and oxygen consumption in healthy premenopausal women was observed (105). Therefore, DNA damage from ROS produced by energy metabolism is a potential cause of natural aging. Whether CR reduces protein, lipid, or DNA damage in nonobese humans has yet to be investigated.

CALORIE RESTRICTION AND CARDIOVASCULAR DISEASE RISK

Atherosclerosis is now recognized as an inflammatory disease (106). The initiating event in the progression of atherosclerosis is

believed to be the development of endothelial dysfunction. Potential causes of endothelial dysfunction include elevated concentrations of oxidatively modified LDL, the generation of free radicals, hypertension, diabetes, and elevated concentrations of homocysteine. The injured endothelium responds to these various insults by developing procoagulant instead of anticoagulant properties and by secreting a number of cytokines and growth factors. The release of these factors leads to the sequestration and accumulation of lymphocytes and macrophages from the blood and to the migration and proliferation of underlying smooth muscle cells. Thus, in addition to the well-recognized cardiovascular disease risk factors including lipids, lipoproteins (LDL and HDL cholesterol and triacylglycerol), and blood pressure, other factors, including hemostasis factors (eg, factor VII, fibrinogen, and plasminogen activator inhibitor type 1), C-reactive protein, and homocysteine, are predictive of cardiovascular disease events (107).

Blood pressure is decreased by CR in the obese (108) and in chronically undernourished laborers (109). Landsberg and Young (110, 111) showed that CR is associated with a decrease in plasma norepinephrine concentration, decreased excretion of catecholamines, and evidence of diminished sympathetic activity. Similar results were found in normal-weight subjects exposed to short-term CR by norepinephrine turnover measures (112). It is likely, therefore, that the decrease in blood pressure during CR is mediated by decreases in insulin concentration and sympathetic nervous activity (113). Short-term CR does not affect concentrations of triacylglycerol or total or LDL cholesterol in nonobese subjects (113, 114), although HDL cholesterol was increased in proportion to the decrease in body weight (113, 115). Long-term CR, on the other hand, was associated with sustained reductions in these factors in nonobese subjects, although HDL-2 concentrations were increased (27). CR may also influence the endothelial function of the vasculature. Recently, Perticone et al (116) reported that endothelial dysfunction, often seen in obese or overweight subjects, is due to oxidative stress (117–119) and can be reversed by acute administration of the potent antioxidant vitamin C. CR also improves endothelium-dependent vasodilatation in obese hypertensive subjects (120). It is therefore logical to hypothesize that CR will improve endothelial function in the nonobese, probably via a decreased production of ROS. CR also reduces markers for inflammation (eg, C-reactive protein, interleukin 6, and plasminogen activator inhibitor type 1) in obese (121–123) and nonobese subjects (124). However, homocysteine concentrations are significantly increased by short-term CR, although this was preventable with vitamin supplementation (125). The effects of long-term CR on markers for inflammation are unknown.

CALORIE RESTRICTION AND INSULIN SENSITIVITY AND SECRETION

Reduced glucose and insulin are hallmark features of CR in rodents and monkeys. There is compelling evidence that CR and the consequent weight loss in the obese (diabetic and nondiabetic alike) greatly improve glucose metabolism by improving insulin action. In a comprehensive review, Kelley (126) concluded that weight loss in obese patients with type 2 diabetes not only reduces fasting hyperglycemia (ie, reduction of postabsorptive hepatic glucose production) but also increases insulin sensitivity (ie, glucose uptake) in peripheral tissues. Whether β cell sensitivity to glucose remains intact with aging is unclear. However, the



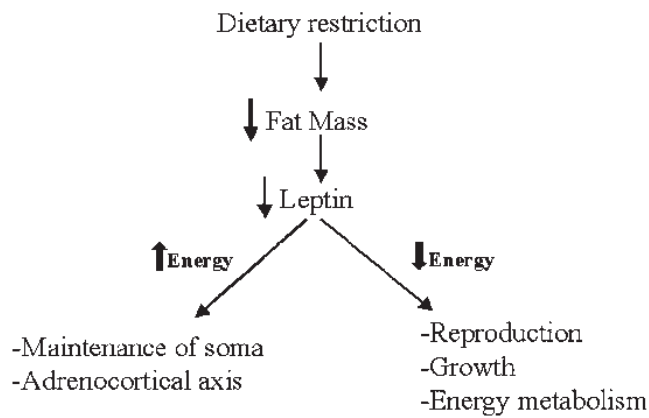


FIGURE 1. Leptin as the master neuroendocrine signal for the anti-aging effects of dietary restriction.

most convincing data that long-term CR is an effective means of avoiding the development of insulin resistance that occurs with aging are from monkey studies (6, 7, 46, 127). Calorie-restricted monkeys had greater insulin sensitivity and increased plasma glucose disappearance rates by the minimal model than did control monkeys at the 8.5-y follow-up (47). Calorie-restricted monkeys also had reduced fasting insulin and a reduced insulin response to glucose. Long-term CR also reduced fasting glucose and insulin concentrations in the lean subjects from the Biosphere 2 experiment (128, 129). Unfortunately, performing more stringent measures of insulin sensitivity was not possible in these subjects.

Whether the improvement in insulin sensitivity is a mechanism by which CR increases life span is a subject of continued debate. It has been proposed that increased insulin and glucose concentrations may contribute to the aging process—insulin because of its mitogenic action (130) and glucose because of protein glycation. This hypothesis, proposed by Masoro and Austad (131), has yet to be tested. However, insulin is known to alter the expression of numerous other hormones, stimulate the sympathetic nervous system, and promote vasoconstriction, all of which could potentially affect longevity. Indeed, fat-specific insulin receptor knockout mice, which have normal or even increased food intake and reduced adiposity, have increased median and maximal life expectancies (132).

In summary, there is evidence that CR in obese and lean subjects alike improves insulin sensitivity. Physiologic mechanisms for this improvement may include decreases in circulating fatty acid concentrations (133), intramyocellular triacylglycerol (79, 134, 135), and secreted cytokines from adipocytes (136–138). Potential molecular mechanisms involved in the relation between fat “at the wrong place” and insulin sensitivity (139), including the ectopic fat hypothesis, which has arisen from the observation that subjects without fat (a condition known as lipodystrophy) have severe insulin resistance (140).

CALORIE RESTRICTION AND THE NEUROENDOCRINE AXES AND AUTONOMIC NERVOUS SYSTEM

The endocrine changes associated with short-term caloric deprivation (CR or starvation) are well described in rodent models, as recently reviewed by Shimokawa and Higami (141). Many of these alterations were described in humans as well and include a drop in triiodothyronine (142), an increase in cortisol secretion

(143), and a decrease in gonadal function. It has long been hypothesized that the neuroendocrine system coordinates and integrates some of the anti-aging actions of CR (35, 36, 144, 145), but little is known about the neuroendocrine pathways that are altered by chronic CR (37, 146–149). One of the major reasons for the paucity of data pertaining to CR and neuroendocrine axes is that neuroendocrine functions are difficult to study in rodents. In a prolonged (48-h) starvation study in mice, Ahima et al (150) provided evidence that the reduction in leptin with starvation caused a decrease in the activity of the gonadal and thyroid axes and an increase in the activity of the adrenal axis. The changes in activity of these axes during fasting were prevented by leptin administration, which suggests a role for leptin as a master regulator of neuroendocrine status. These results support the disposable soma theory on the evolution of aging, which states that longevity requires investment in somatic maintenance by reducing the resources available for reproduction (141, 151). Down-regulation of neuroendocrine activity has been interpreted as a marker of somatic preservation, and leptin has been suggested as the candidate endocrine mediator for this effect (**Figure 1**). In subjects with congenital acquired lipodystrophy (the absence of fat and leptin), the administration of exogenous recombinant leptin normalizes the metabolic milieu (152, 153). Furthermore, the administration of “replacement” doses of leptin in obese and nonobese subjects reverses the reductions in triiodothyroxine, thyroxine, and TEE that are normally observed after 10% weight loss (154). These studies suggest that leptin coordinates many of the neuroendocrine actions of CR in humans.


There is also considerable evidence that the growth hormone (GH)–insulin-like growth factor I (IGF-I) axis may mediate some of the effects of CR. In *C. elegans*, the loss of functional mutations in the insulin–IGF-I signaling pathway nearly doubled the expected life span (155). Further studies in genetically altered mice (eg, Ames dwarf mouse, Snell dwarf mouse, GH-receptor knockout mouse) show that changes in GH secretion (leading to changes in IGF-I production) delay aging and prolong life span (156). However, these animals have numerous other endocrine defects that confound these results. Recently, IGF-I knockout mice were shown to live 26% longer than wild-type littermates. These animals did not develop dwarfism, had normal energy metabolism, and had greater resistance to oxidative stress (157). The pygmy population in the Philippines also has an altered GH–IGF-I axis, with reduced concentrations of IGF-I, growth hormone-binding protein, and IGF-binding protein-3 compared with Philippine control subjects (158). However, whether any of the pygmy populations have increased longevity is unknown. Further studies are required to delineate the role of leptin, the GH–IGF-I axes, and the thyroid axes in altering markers of aging during prolonged CR in nonobese humans.

CALORIE RESTRICTION AND GENE EXPRESSION

Gene expression profiling with the use of DNA microarrays has revealed that aging is associated with several alterations in gene expression in rodent skeletal muscle (142), brain (159), and heart (160) and that CR prevents many of these changes. Transcriptional patterns suggest that CR retards aging by causing a metabolic shift toward increased protein turnover and decreased macromolecular damage (161). Several of these genes are also dysregulated during aging in humans (162).

Recently, skeletal muscle gene expression in aged primates was compared with that in young animals (48). Aging selectively up-regulated transcripts involved in inflammation and oxidative stress and down-regulated genes involved in mitochondrial electron transport and oxidative phosphorylation. CR up-regulated cytoskeletal protein-encoding genes and decreased the expression of genes involved in mitochondrial bioenergetics, but, surprisingly, the inhibitory effect of CR on age-related changes in gene expression was not observed (48). The effects of prolonged CR on gene expression profiles in human skeletal muscle and adipose tissue are unknown.

FUTURE STUDIES IN HUMANS

As reviewed here, CR leads to numerous changes in animal models, including alterations in body composition, EE, oxidative damage, cardiovascular disease, insulin sensitivity, neuroendocrine function, and gene expression. Because of the pluripotent nature of CR, the mechanism or mechanisms by which CR extends life span are still very much debated. Furthermore, it is not known whether CR extends longevity in long-lived species. Randomized controlled trials investigating the effects and possible mechanisms of prolonged CR in nonobese humans are long overdue. The clinical trial named CALERIE (Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy), funded by the National Institute of Aging and initiated in 2002, will address this gap by examining the effects of chronic CR on surrogate markers for longevity in nonobese humans. 

REFERENCES

- McCay CM, Crowell MF, Maynard LA. The effect of retarded growth upon the length of the life span and upon the ultimate body size. *J Nutr* 1935;10:63–79.
- Barrows CH, Kokkonen GC. Dietary restriction and life extension, biological mechanisms. In: Moment GB, ed. *Nutritional approaches to aging research*. Boca Raton, FL: CRC Press Inc, 1982:219–43.
- Weindruch R, Walford RL. *The retardation of aging and disease by dietary restriction*. Springfield, IL: Charles C Thomas Publisher, 1988.
- Roth GS, Lane MA, Ingram DK, et al. Biomarkers of caloric restriction may predict longevity in humans. *Science* 2002;297:811.
- 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.
- Bodkin NL, Ortmeier HK, Hansen BC. Long-term dietary restriction in older-aged rhesus monkeys: effects on insulin resistance. *J Gerontol A Biol Sci Med Sci* 1995;50:B142–7.
- Lane MA, Black A, Ingram DK, Roth GS. Calorie restriction in non-human primates: implications for age-related disease risk. *J Anti-Aging Med* 1998;1:315–26.
- Mazat L, Lafont S, Berr C, et al. Prospective measurements of dehydroepiandrosterone sulfate in a cohort of elderly subjects: relationship to gender, subjective health, smoking habits, and 10-year mortality. *Proc Natl Acad Sci U S A* 2001;98:8145–50.
- Ravaglia G, Forti P, Maioli F, et al. Dehydroepiandrosterone-sulfate serum levels and common age-related diseases: results from a cross-sectional Italian study of a general elderly population. *Exp Gerontol* 2002;37:701–12.
- Kahonen MH, Tilvis RS, Jolkkonen J, Pitkala K, Harkonen M. Predictors and clinical significance of declining plasma dehydroepiandrosterone sulfate in old age. *Aging (Milano)* 2000;12:308–14.
- Committee on a National Research Agenda on Aging. *Extending life, enhancing life: a national research agenda on aging*. Washington, DC: National Academy Press, 1991.
- Schneider EL, Vining EM, Hadley EC, Farnham SA. Recommended dietary allowances and the health of the elderly. *N Engl J Med* 1986;314:157–60.
- Eveleth PB, Tanner JM. *Worldwide variation in human growth*. 2nd ed. New York: Cambridge University Press, 1990.
- Ellison PT. Developmental influences on adult ovarian function. *Am J Hum Biol* 1996;8:725–34.
- Ellison PT. Age and developmental effects on adult ovarian function. In: Mascie-Taylor NCG, ed. *Variability in human fertility: a biological anthropological approach*. New York: Cambridge University Press, 1996:69–90.
- Ellison PT, Panter-Brick C, Lipson SF, O'Rourke MT. The ecological context of human ovarian function. *Hum Reprod* 1993;8:2248–58.
- Roberts SB, Paul AA, Cole TJ, Whitehead RG. Seasonal changes in activity, birth weight and lactational performance in rural Gambian women. *Trans R Soc Trop Med Hyg* 1982;76:668–78.
- Lee RB. *The !Kung San: men, women, and work in a foraging society*. Chicago: University of Chicago Press, 1979.
- Martorell R. Interrelationships between diet, infectious disease, and nutritional status. In: Greene LS, Johnston FE, eds. *Social and biological predictors of nutritional status, physical growth, and neurological development*. New York: Academic Press, 1980:81–106.
- Ulijaszek SJ. Nutritional status and susceptibility to infectious disease. In: Waterlow JC, ed. *Diet and disease*. New York: Cambridge University Press, 1990:137–54.
- Keys A, Brozek J, Henschel A, Mickelson O, Taylor H. *The biology of human starvation*. Minneapolis: University of Minnesota Press, 1950.
- Kagawa Y. Impact of westernization on the nutrition of Japanese: changes in physique, cancer, longevity and centenarians. *Prev Med* 1978;7:205–17.
- Mizushima S, Yopori Y. Nutritional improvement, cardiovascular diseases and longevity in Japan. *Nutr Health* 1992;8:97–105.
- Vallejo EA. La dieta de hambre a dias alternos in la alimentacion de los viejos. (Fasting on alternate days in the feeding of the elderly.) *Rev Clin Exp* 1957;63:25–6 (in Spanish).
- Stunkard AJ. Nutrition, aging and obesity. In: Rockstein M, Sussman ML, eds. *Nutrition, longevity, and aging*. New York: Academic Press, 1976:253–84.
- Weyer C, Walford RL, Harper IT, et al. Energy metabolism after 2 y of energy restriction: the Biosphere 2 experiment. *Am J Clin Nutr* 2000;71:946–53.
- Walford RL, Mock D, Verdery R, MacCallum T. Calorie restriction in Biosphere 2: alterations in physiologic, hematologic, hormonal, and biochemical parameters in humans restricted for a 2-year period. *J Gerontol A Biol Sci Med Sci* 2002;57:B211–24.
- Holloszy JO, Smith EK, Vining M, Adams S. Effect of voluntary exercise on longevity of rats. *J Appl Physiol* 1985;59:826–31.
- Holloszy JO. Mortality rate and longevity of food-restricted exercising male rats: a reevaluation. *J Appl Physiol* 1997;82:399–403.
- Blair SN, Kohl HW 3d, Paffenbarger RS Jr, Clark DG, Cooper KH, Gibbons LW. Physical fitness and all-cause mortality. A prospective study of healthy men and women. *JAMA* 1989;262:2395–401.
- Villeneuve PJ, Morrison HI, Craig CL, Schaubel DE. Physical activity, physical fitness, and risk of dying. *Epidemiology* 1998;9:626–31.
- Ballor DL. Effect of dietary restriction and/or exercise on 23-h metabolic rate and body composition in female rats. *J Appl Physiol* 1991;71:801–6.
- Havel PJ. Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. *Curr Opin Lipidol* 2002;13:51–9.
- Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. *Science* 1996;273:59–63.



35. Meites J. Evidence that underfeeding acts via the neuroendocrine system to influence aging processes. *Prog Clin Biol Res* 1989;287:169–80.
36. Nelson JF. Neuroendocrine involvement in the retardation of aging by food restriction: A hypothesis. In: Yu BP, ed. *Modulation of aging processes by dietary restriction*. Boca Raton, FL: CRC Press, 1994:37–55.
37. Armario A, Montero JL, Jolin T. Chronic food restriction and the circadian rhythms of pituitary-adrenal hormones, growth hormone and thyroid-stimulating hormone. *Ann Nutr Metab* 1987;31:81–7.
38. Lee CK, Klopp RG, Weindrich R, Prolla TA. Gene expression profile of aging and its retardation by caloric restriction. *Science* 1999;285:1390–3.
39. Russell JC, Epling WF, Pierce D, Amy RM, Boer DP. Induction of voluntary prolonged running by rats. *J Appl Physiol* 1987;63:2549–53.
40. Duffy PH, Feuers R, Nakamura KD, Leakey J, Hart RW. Effect of chronic caloric restriction on the synchronization of various physiological measures in old female Fischer 344 rats. *Chronobiol Int* 1990;7:113–24.
41. Girard N, Ferland G, Boulanger L, Gaudreau P. Long-term calorie restriction protects rat pituitary growth hormone-releasing hormone binding sites from age-related alterations. *Neuroendocrinology* 1998;68:21–9.
42. Herlihy JT, Stacy C, Bertrand HA. Long-term calorie restriction enhances baroreflex responsiveness in Fischer 344 rats. *Am J Physiol* 1992;263:H1021–5.
43. McShane T, Wise P. Life-long moderate caloric restriction prolongs reproductive life span in rats without interrupting estrous cyclicity: effects on the gonadotropin-releasing hormone/luteinizing hormone axis. *Biol Reprod* 1996;54:70–5.
44. Ravussin E, Lillioja S, Anderson TE, Christin L, Bogardus C. Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. *J Clin Invest* 1986;78:1568–78.
45. Gonzales-Pacheco DM, Buss WC, Koehler KM, Woodside WF, Alpert SS. Energy restriction reduces metabolic rate in adult male Fisher-344 rats. *J Nutr* 1993;123:90–7.
46. Cefalu WT, Wagner JD, Bell-Farrow AD, et al. Influence of caloric restriction on the development of atherosclerosis in nonhuman primates: progress to date. *Toxicol Sci* 1999;52:49–55.
47. Gresl TA, Colman RJ, Roecker EB, et al. Dietary restriction and glucose regulation in aging rhesus monkeys: a follow-up report at 8.5 yr. *Am J Physiol Endocrinol Metab* 2001;281:E757–65.
48. Kayo T, Allison DB, Weindrich R, Prolla TA. Influences of aging and caloric restriction on the transcriptional profile of skeletal muscle from rhesus monkeys. *Proc Natl Acad Sci U S A* 2001;98:5093–8.
49. Roth GS, Ingram DK, Black A, Lane MA. Effects of reduced energy intake on the biology of aging: the primate model. *Eur J Clin Nutr* 2000;54:S15–S20.
50. Lane MA, Black A, Handy AM, et al. Energy restriction does not alter bone mineral metabolism or reproductive cycling and hormones in female rhesus monkeys. *J Nutr* 2001;131:820–7.
51. Lane MA, Tilmont EM, De Angelis H, et al. Short-term calorie restriction improves disease-related markers in older male rhesus monkeys (*Macaca mulatta*). *Mech Ageing Dev* 2000;112:185–96.
52. Weindrich R, Walford RL. Dietary restriction in mice beginning at 1 year of age: effect on life-span and spontaneous cancer incidence. *Science* 1982;215:1415–8.
53. Sacher GA. Life table modifications and life prolongation. In: Hayflick L, ed. *Handbook of the biology of aging*. New York: Van Nostrand Reinold, 1977:582–638.
54. Sacher GA, Duffy PH. Genetic relation of life span to metabolic rate for inbred mouse strains and their hybrids. *Fed Proc* 1979;38:184–8.
55. Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert Consultation. *World Health Organ Tech Rep Ser* 1985;724:1–206.
56. McCarter R, Masoro EJ, Yu BP. Does food restriction retard aging by reducing the metabolic rate? *Am J Physiol* 1985;248:E488–90.
57. McCarter RJ, Palmer J. Energy metabolism and aging: a lifelong study of Fischer 344 rats. *Am J Physiol* 1992;263:E448–52.
58. Lynn WS, Wallwork JC. Does food restriction retard aging by reducing metabolic rate? *J Nutr* 1992;122:1917–8.
59. Blanc S, Schoeller D, Kemnitz J, et al. Energy expenditure of rhesus monkeys subjected to 11 years of dietary restriction. *J Clin Endocrinol Metab* 2003;88:16–23.
60. Poehlman ET, Melby CL, Badylak SF. Relation of age and physical exercise status on metabolic rate in younger and older healthy men. *J Gerontol* 1991;46:B54–8.
61. Ramsey JJ, Roecker EB, Weindrich R, Kemnitz JW. Energy expenditure of adult male rhesus monkeys during the first 30 mo of dietary restriction. *Am J Physiol* 1997;272:E901–7.
62. DeLany JP, Hansen BC, Bodkin NL, Hannah J, Bray GA. Long-term calorie restriction reduces energy expenditure in aging monkeys. *J Gerontol A Biol Sci Med Sci* 1999;54:B5–11; discussion B12–3.
63. Rising R, Keys A, Ravussin E, Bogardus C. Concomitant interindividual variation in body temperature and metabolic rate. *Am J Physiol* 1992;263:E730–4.
64. Fricker J, Rozen R, Melchior JC, Apfelbaum M. Energy-metabolism adaptation in obese adults on a very-low-calorie diet. *Am J Clin Nutr* 1991;53:826–30.
65. Vansant G, Van Gaal L, Van Acker K, De Leeuw I. Short and long term effects of a very low calorie diet on resting metabolic rate and body composition. *Int J Obes* 1989;13:87–9.
66. Keesey RE. Physiological regulation of body weight and the issue of obesity. *Med Clin North Am* 1989;73:15–27.
67. Astrup A, Gotzsche PC, van de Werken K, et al. Meta-analysis of resting metabolic rate in formerly obese subjects. *Am J Clin Nutr* 1999;69:1117–22.
68. Leibel RL, Rosenbaum M, Hirsch J. Changes in energy expenditure resulting from altered body weight. *N Engl J Med* 1995;332:621–8.
69. Weigle DS, Sande KJ, Iverius PH, Monsen ER, Brunzell JD. Weight loss leads to a marked decrease in nonresting energy expenditure in ambulatory human subjects. *Metabolism* 1988;37:930–6.
70. Astrup A, Buemann B, Toubro S, Ranneries C, Raben A. Low resting metabolic rate in subjects predisposed to obesity: a role for thyroid status. *Am J Clin Nutr* 1996;63:879–83.
71. Weigle DS, Brunzell JD. Assessment of energy expenditure in ambulatory reduced-obese subjects by the techniques of weight stabilization and exogenous weight replacement. *Int J Obes* 1990;14:69–77; discussion 77–81.
72. Rosenbaum M, Ravussin E, Matthews DE, et al. A comparative study of different means of assessing long-term energy expenditure in humans. *Am J Physiol* 1996;270:R496–504.
73. Barzilai N, Gupta G. Interaction between aging and syndrome X: new insights on the pathophysiology of fat distribution. *Ann NY Acad Sci* 1999;892:58–72.
74. Cefalu WT, Wagner JD, Wang ZQ, et al. A study of caloric restriction and cardiovascular aging in cynomolgus monkeys (*Macaca fascicularis*): a potential model for aging research. *J Gerontol A Biol Sci Med Sci* 1997;52:B10–9.
75. Bertrand HA, Lynd FT, Masoro EJ, Yu BP. Changes in adipose mass and cellularity through the adult life of rats fed ad libitum or a life-prolonging restricted diet. *J Gerontol* 1980;35:827–35.
76. Gabriely I, Ma XH, Yang XM, et al. Removal of visceral fat prevents insulin resistance and glucose intolerance of aging: an adipokine-mediated process? *Diabetes* 2002;51:2951–8.
77. Barzilai N, Banerjee S, Hawkins M, Chen W, Rossetti L. Caloric restriction reverses hepatic insulin resistance in aging rats by decreasing visceral fat. *J Clin Invest* 1998;101:1353–61.
78. Frayn KN. Visceral fat and insulin resistance—causative or correlative? *Br J Nutr* 2000;83(suppl):S71–7.



79. Jacob S, Machann J, Rett K, et al. Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. *Diabetes* 1999;48:1113–9.
80. Goodpaster BH, Kelley DE, Wing RR, Meier A, Thaete FL. Effects of weight loss on regional fat distribution and insulin sensitivity in obesity. *Diabetes* 1999;48:839–47.
81. Houmard JA, Tanner CJ, Yu C, et al. Effect of weight loss on insulin sensitivity and intramuscular long-chain fatty acyl-CoAs in morbidly obese subjects. *Diabetes* 2002;51:2959–63.
82. Sohal RS, Allen RG. Relationship between metabolic rate, free radicals, differentiation and aging: a unified theory. *Basic Life Sci* 1985;35:75–104.
83. Orr WC, Sohal RS. Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. *Science* 1994;263:1128–30.
84. Melov S, Ravenscroft J, Malik S, et al. Extension of life-span with superoxide dismutase/catalase mimetics. *Science* 2000;289:1567–9.
85. Lee DW, Yu BP. Modulation of free radicals and superoxide dismutases by age and dietary restriction. *Aging (Milano)* 1990;2:357–62.
86. Parkes TL, Elia AJ, Dickinson D, Hilliker AJ, Phillips JP, Boulianne GL. Extension of *Drosophila* lifespan by overexpression of human SOD1 in motoneurons. *Nat Genet* 1998;19:171–4.
87. Migliaccio E, Giorgio M, Mele S, et al. The p66shc adaptor protein controls oxidative stress response and life span in mammals. *Nature* 1999;402:309–13.
88. Chevion M, Berenshtein E, Stadtman ER. Human studies related to protein oxidation: protein carbonyl content as a marker of damage. *Free Radic Res* 2000;33(suppl):S99–108.
89. Dandona P, Mohanty P, Ghanim H, et al. The suppressive effect of dietary restriction and weight loss in the obese on the generation of reactive oxygen species by leukocytes, lipid peroxidation, and protein carbonylation. *J Clin Endocrinol Metab* 2001;86:355–62.
90. Pratico D, Barry OP, Lawson JA, et al. IPF2alpha-I: an index of lipid peroxidation in humans. *Proc Natl Acad Sci U S A* 1998;95:3449–54.
91. Morrow JD, Frei B, Longmire AW, et al. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. *N Engl J Med* 1995;332:1198–203.
92. Aleynik SI, Leo MA, Aleynik MK, Lieber CS. Increased circulating products of lipid peroxidation in patients with alcoholic liver disease. *Alcohol Clin Exp Res* 1998;22:192–6.
93. Delanty N, Reilly MP, Pratico D, et al. 8-epi PGF2 alpha generation during coronary reperfusion. A potential quantitative marker of oxidant stress in vivo. *Circulation* 1997;95:2492–9.
94. Montine TJ, Markesbery WR, Morrow JD, Roberts LJ 2nd. Cerebrospinal fluid F2-isoprostane levels are increased in Alzheimer's disease. *Ann Neurol* 1998;44:410–3.
95. Pratico D, Basili S, Vieri M, Cordova C, Violi F, Fitzgerald GA. Chronic obstructive pulmonary disease is associated with an increase in urinary levels of isoprostane F2alpha-III, an index of oxidant stress. *Am J Respir Crit Care Med* 1998;158:1709–14.
96. Wang Z, Ciabattoni G, Creminon C, et al. Immunological characterization of urinary 8-epi-prostaglandin F2 alpha excretion in man. *J Pharmacol Exp Ther* 1995;275:94–100.
97. Davi G, Guagnano MT, Ciabattoni G, et al. Platelet activation in obese women: role of inflammation and oxidant stress. *JAMA* 2002;288:2008–14.
98. Bohr VA, Dianov GL. Oxidative DNA damage processing in nuclear and mitochondrial DNA. *Biochimie* 1999;81:155–60.
99. Adelman R, Saul RL, Ames BN. Oxidative damage to DNA: relation to species metabolic rate and life span. *Proc Natl Acad Sci U S A* 1988;85:2706–8.
100. Dizdaroglu M, Nackerdien Z, Chao BC, Gajewski E, Rao G. Chemical nature of in vivo DNA base damage in hydrogen peroxide-treated mammalian cells. *Arch Biochem Biophys* 1991;285:388–90.
101. Loeb LA, Preston BD. Mutagenesis by apurinic/apyrimidinic sites. *Annu Rev Genet* 1986;20:201–30.
102. Sagher D, Strauss B. Insertion of nucleotides opposite apurinic/apyrimidinic sites in deoxyribonucleic acid during in vitro synthesis: uniqueness of adenine nucleotides. *Biochemistry* 1983;22:4518–26.
103. Fraga CG, Shigenaga MK, Park JW, Degan P, Ames BN. Oxidative damage to DNA during aging: 8-hydroxy-2'-deoxyguanosine in rat organ DNA and urine. *Proc Natl Acad Sci U S A* 1990;87:4533–7.
104. Atamna H, Cheung I, Ames BN. A method for detecting abasic sites in living cells: age-dependent changes in base excision repair. *Proc Natl Acad Sci U S A* 2000;97:686–91.
105. Loft S, Astrup A, Buemann B, Poulsen HE. Oxidative DNA damage correlates with oxygen consumption in humans. *FASEB J* 1994;8:534–7.
106. Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med* 1999;340:115–26.
107. Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. *JAMA* 2001;285:2481–5.
108. Apfelbaum M. Adaptation to changes in caloric intake. *Prog Food Nutr Sci* 1978;2:543–59.
109. Shetty PS, Kurpad AV. Role of the sympathetic nervous system in adaptation to seasonal energy deficiency. *Eur J Clin Nutr* 1990;44:47–53.
110. Landsberg L, Young JB. Diet-induced changes in sympathetic nervous system activity. In: Bassett EG, ed. *Nutritional factors: modulating effects on metabolic processes*. New York: Raven Press, 1981: 155–74.
111. Landsberg L, Young JB. The role of the sympathetic nervous system and catecholamines in the regulation of energy metabolism. *Am J Clin Nutr* 1983;38:1018–24.
112. O'Dea K, Esler M, Leonard P, Stockigt JR, Nestel P. Noradrenaline turnover during under- and over-eating in normal weight subjects. *Metabolism* 1982;31:896–9.
113. Velthuis-te Wierik EJ, van den Berg H, Schaafsma G, Hendriks HF, Brouwer A. Energy restriction, a useful intervention to retard human ageing? Results of a feasibility study. *Eur J Clin Nutr* 1994;48: 138–48.
114. Wood PD, Stefanick ML, Williams PT, Haskell WL. The effects on plasma lipoproteins of a prudent weight-reducing diet, with or without exercise, in overweight men and women. *N Engl J Med* 1991; 325:461–6.
115. Dattilo AM, Kris-Etherton PM. Effects of weight reduction on blood lipids and lipoproteins: a meta-analysis. *Am J Clin Nutr* 1992;56:320–8.
116. Peticone F, Ceravolo R, Candigliota M, et al. Obesity and body fat distribution induce endothelial dysfunction by oxidative stress: protective effect of vitamin C. *Diabetes* 2001;50:159–65.
117. Ting HH, Timimi FK, Haley EA, Roddy MA, Ganz P, Creager MA. Vitamin C improves endothelium-dependent vasodilation in forearm resistance vessels of humans with hypercholesterolemia. *Circulation* 1997;95:2617–22.
118. Heitzer T, Just H, Munzel T. Antioxidant vitamin C improves endothelial dysfunction in chronic smokers. *Circulation* 1996;94:6–9.
119. Taddei S, Virdis A, Ghiadoni L, Magagna A, Salvetti A. Vitamin C improves endothelium-dependent vasodilation by restoring nitric oxide activity in essential hypertension. *Circulation* 1998;97:2222–9.
120. Sasaki S, Higashi Y, Nakagawa K, et al. A low-calorie diet improves endothelium-dependent vasodilation in obese patients with essential hypertension. *Am J Hypertens* 2002;15:302–9.
121. Heilbronn LK, Noakes M, Clifton PM. Energy restriction and weight loss on very-low-fat diets reduce C-reactive protein concentrations in obese, healthy women. *Arterioscler Thromb Vasc Biol* 2001;21: 968–70.
122. Bastard JP, Jardel C, Bruckert E, et al. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J Clin Endocrinol Metab* 2000;85:3338–42.



123. Mavri A, Alessi MC, Bastelica D, et al. Subcutaneous abdominal, but not femoral fat expression of plasminogen activator inhibitor-1 (PAI-1) is related to plasma PAI-1 levels and insulin resistance and decreases after weight loss. *Diabetologia* 2001;44:2025–31.
124. Velthuis-te Wierik EJ, Meijer P, Kluft C, van den Berg H. Beneficial effect of a moderately energy-restricted diet on fibrinolytic factors in non-obese men. *Metabolism* 1995;44:1548–52.
125. Volek JS, Gomez AL, Love DM, et al. Effects of an 8-week weight-loss program on cardiovascular disease risk factors and regional body composition. *Eur J Clin Nutr* 2002;56:585–92.
126. Kelley DE. Effects of weight loss on glucose homeostasis in NIDDM. *Diabetes Rev* 1995;3:366–77.
127. Gazdag AC, Sullivan S, Kemnitz JW, Cartee GD. Effect of long-term caloric restriction on GLUT4, phosphatidylinositol-3 kinase p85 subunit, and insulin receptor substrate-1 protein levels in rhesus monkey skeletal muscle. *J Gerontol A Biol Sci Med Sci* 2000;55:B44–6; discussion B47–8.
128. Walford RL, Mock D, MacCallum T, Laseter JL. Physiologic changes in humans subjected to severe, selective calorie restriction for two years in Biosphere 2: health, aging, and toxicological perspectives. *Toxicol Sci* 1999;52:61–5.
129. Walford RL, Weber L, Panov S. Caloric restriction and aging as viewed from Biosphere 2. *Receptor* 1995;5:29–33.
130. Lev-Ran A. Mitogenic factors accelerate later-age diseases: insulin as a paradigm. *Mech Ageing Dev* 1998;102:95–113.
131. Masoro EJ, Austad SN. The evolution of the antiaging action of dietary restriction: a hypothesis. *J Gerontol A Biol Sci Med Sci* 1996;51:B387–91.
132. Bluher M, Kahn BB, Kahn CR. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 2003;299:572–4.
133. Boden G. Free fatty acids—the link between obesity and insulin resistance. *Endocr Pract* 2001;7:44–51.
134. Forouhi NG, Jenkinson G, Thomas EL, et al. Relation of triglyceride stores in skeletal muscle cells to central obesity and insulin sensitivity in European and South Asian men. *Diabetologia* 1999;42:932–5.
135. Perseghin G, Scifo P, De Cobelli F, et al. Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a ¹H-¹³C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes* 1999;48:1600–6.
136. Flier JS. Diabetes. The missing link with obesity? *Nature* 2001;409:292–3.
137. Simpson HL, Umpleby AM, Russell-Jones DL. Insulin-like growth factor-I and diabetes. A review. *Growth Horm IGF Res* 1998;8:83–95.
138. Lofgren P, van Harmelen V, Reynisdottir S, et al. Secretion of tumor necrosis factor- α shows a strong relationship to insulin-stimulated glucose transport in human adipose tissue. *Diabetes* 2000;49:688–92.
139. Shulman GI. Cellular mechanisms of insulin resistance. *J Clin Invest* 2000;106:171–6.
140. Ravussin E, Smith SR. Increased fat intake, impaired fat oxidation, and failure of fat cell proliferation result in ectopic fat storage, insulin resistance, and type 2 diabetes mellitus. *Ann NY Acad Sci* 2002;967:363–78.
141. Shimokawa I, Higami Y. Leptin and anti-aging action of caloric restriction. *J Nutr Health Aging* 2001;5:43–8.
142. Roti E, Minelli R, Salvi M. Thyroid hormone metabolism in obesity. *Int J Obes Relat Metab Disord* 2000;24(suppl):S113–5.
143. Fichter MM, Pirke KM, Holsboer F. Weight loss causes neuroendocrine disturbances: experimental study in healthy starving subjects. *Psychiatry Res* 1986;17:61–72.
144. Everitt AV, Seedsman NJ, Jones F. The effects of hypophysectomy and continuous food restriction, begun at ages 70 and 400 days, on collagen aging, proteinuria, incidence of pathology and longevity in the male rat. *Mech Ageing Dev* 1980;12:161–72.
145. Masoro EJ. Food restriction in rodents: an evaluation of its role in the study of aging. *J Gerontol* 1988;43:B59–64.
146. Klebanov S, Diais S, Stavinoha WB, Suh Y, Nelson JF. Hyperadrenocorticism, attenuated inflammation, and the life-prolonging action of food restriction in mice. *J Gerontol A Biol Sci Med Sci* 1995;50:B79–82.
147. Herlihy JT, Stacy C, Bertrand HA. Long-term food restriction depresses serum thyroid hormone concentrations in the rat. *Mech Ageing Dev* 1990;53:9–16.
148. Sabatino F, Masoro EJ, McMahan CA, Kuhn RW. Assessment of the role of the glucocorticoid system in aging processes and in the action of food restriction. *J Gerontol* 1991;46:B171–9.
149. Sonntag WE, Lynch CD, Cefalu WT, et al. Pleiotropic effects of growth hormone and insulin-like growth factor (IGF)-1 on biological aging: inferences from moderate caloric-restricted animals. *J Gerontol A Biol Sci Med Sci* 1999;54:B521–38.
150. Ahima RS, Prabakaran D, Mantzoros C, et al. Role of leptin in the neuroendocrine response to fasting. *Nature* 1996;382:250–2.
151. Kirkwood TB. Evolution of ageing. *Nature* 1977;270:301–4.
152. Simha V, Szczeniak LS, Wagner AJ, DePaoli AM, Garg A. Effect of leptin replacement on intrahepatic and intramyocellular lipid content in patients with generalized lipodystrophy. *Diabetes Care* 2003;26:30–5.
153. Petersen KF, Oral EA, Dufour S, et al. Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. *J Clin Invest* 2002;109:1345–50.
154. Rosenbaum M, Murphy EM, Heymsfield SB, Matthews DE, Leibel RL. Low dose leptin administration reverses effects of sustained weight-reduction on energy expenditure and circulating concentrations of thyroid hormones. *J Clin Endocrinol Metab* 2002;87:2391–4.
155. Lin K, Hsin H, Libina N, Kenyon C. Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. *Nat Genet* 2001;28:139–45.
156. Bartke A, Brown-Borg H, Mattison J, Kinney B, Hauck S, Wright C. Prolonged longevity of hypopituitary dwarf mice. *Exp Gerontol* 2001;36:21–8.
157. Holzenberger M, Dupont J, Ducos B, et al. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* 2003;421:182–7.
158. Davila N, Shea BT, Omoto K, Mercado M, Misawa S, Baumann G. Growth hormone binding protein, insulin-like growth factor-I and short stature in two pygmy populations from the Philippines. *J Pediatr Endocrinol Metab* 2002;15:269–76.
159. Lee CK, Weindruch R, Prolla TA. Gene-expression profile of the ageing brain in mice. *Nat Genet* 2000;25:294–7.
160. Lee C-K, Allison DB, Brand J, Weindruch R, Prolla TA. Transcriptional profiles associated with aging and middle age-onset caloric restriction in mouse hearts. *Proc Natl Acad Sci U S A* 2002;99:14988–93.
161. Weindruch R, Kayo T, Lee CK, Prolla TA. Gene expression profiling of aging using DNA microarrays. *Mech Ageing Dev* 2002;123:177–93.
162. Welle S, Brooks A, Thornton CA. Senescence-related changes in gene expression in muscle: similarities and differences between mice and men. *Physiol Genomics* 2001;5:67–73.

