FEBS Letters 585 (2011) 1537–1542

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

Calorie restriction and prevention of age-associated chronic disease

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ARTICLE INFO

Article history:
Received 22 February 2011
Revised 4 March 2011
Accepted 7 March 2011
Available online 12 March 2011

Edited by Sergio Papa, Gianfranco Gilardi and Wilhelm Just

Keywords:
Calorie restriction
Aging
Chronic disease
Prevention

ABSTRACT

Life expectancy in the world has increased dramatically during the last century; the number of older adults is expected to rise while the number of youths will decline in the near future. This demographic shift has considerable public health and economic implications since aging is associated with the development of serious chronic diseases. Calorie restriction (CR) is the most effective nutritional intervention for slowing aging and preventing chronic disease in rodents. In non-human and human primates, CR with adequate nutrition protects against abdominal obesity, diabetes, hypertension and cardiovascular diseases. Cancer morbidity and mortality are also diminished in CR monkeys, and data obtained from individuals practicing long-term CR show a reduction of metabolic and hormonal factors associated with increased cancer risk.

1. Introduction

In the last century life expectancy at birth has markedly increased from about 45 years at the beginning of the 20th Century to about 77 years today in many developed countries, including Western Europe, USA, Canada, Japan, Australia, and New Zealand [1]. This increase is due primarily to reduced infant mortality, better hygiene, improved sanitation, the development of antibiotics and vaccines, and better healthcare [2]. However, the overall increase in average lifespan is far greater than that for healthy lifespan, as evidenced by the rising burden of chronic diseases, including abdominal obesity, type 2 diabetes, chronic lower respiratory disease, Alzheimer's disease, heart and cerebrovascular diseases, and malignant neoplasms [3]. Approximately 80% of older adults (+65 years) have at least one of the above mentioned chronic diseases, and 50% have at least two chronic diseases [4]. Major risk factors for the onset of some of the most prevalent chronic diseases are the consumption of diets rich in empty calories and poor in nutrients (e.g., vitamins, phytochemicals), physical inactivity and smoking; unless there are substantial reductions in the underlying risk factors, the human and economic costs from cardiovascular disease (CVD), cancer and diabetes are expected to rise in the near future. In contrast to the detrimental effects of overeating energy-dense foods, a reduction in calorie intake without malnutrition defined as calorie restriction (CR), has a wide range of benefits. Moderate CR can prevent or reverse the damaging effects of overweight/obesity, type 2 diabetes, hypertension, chronic inflammation and other age-associated metabolic diseases. Studies on rodents, monkeys, and preliminary studies on humans have shown that more severe CR has additional benefits. The purpose of this article is to review the current knowledge on the effects of CR on disease risk and life expectancy in model organisms and humans.

2. Calorie restriction in model organisms

CR remains the most robust non-genetic nutritional experimental intervention for life extension in many species, including yeast, fruit flies, nematodes, fish, rats, mice, and dogs [5,6]. Invertebrate model organisms (i.e., yeast, *Caenorhabditis elegans*, and *Drosophila*) are well-suited for the analysis of the molecular anti-aging mechanisms of CR due to their relative simplicity and shorter time needed to complete longevity studies, as discussed in more detail elsewhere [7,8]. However, the metabolic, anatomical, physiological and lifespan differences between these invertebrate model organisms and the mammalian systems are enormous. Rodents provide an extremely valuable and flexible animal model in which to determine the ability of CR to extend maximum lifespan and healthspan in a mammalian system. In rodents, a 30–60% reduction in calorie intake below usual ad libitum intake initiated early in life caused a

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doi:10.1016/j.febslet.2011.03.015

proportionate 30–60% increase in maximum life span [5,6]. CR started in adulthood (age 12 months) extended maximum life span by only 10–20% [9]. To date, mice and rats are the only mammals in which CR has clearly been shown to increase both average and maximal lifespan, and to decelerate many age-dependent physiological and structural changes in multiple organs and tissues. In addition, data from studies with laboratory rodents found that CR without malnutrition increases healthspan by preventing or delaying the occurrence of a wide range of chronic diseases. In rodents, cancer is the leading cause of death, accounting for 70–80% of all deaths, and CR has been shown to inhibit spontaneous, chemically-induced and radiation-induced tumors in several murine models of cancer [10]. CR without malnutrition has also been shown to prevent or delay the occurrence of chronic nephropathies and cardiomyopathies, the second leading causes of death in rodents [5,11]. Moreover, in ApoE knockout mice CR reduced the size and progression of atherosclerotic lesions when compared to ApoE−/− mice fed ad libitum, which developed more advanced and fibrotic lesions [12]. Diabetes, autoimmune and respiratory disease are also prevented by CR [5,6]. Finally, CR in mice decreases neurodegeneration, β-amyloid deposition in the brain and enhances neurogenesis in animal models of Alzheimer disease, Parkinson disease, Huntington disease, and stroke [13,14]. However, under certain conditions chronic CR may also potentially impair some key functions, such as immunity and wound healing. For example, the healing of skin wounds is reduced in long-term CR mice, but is greatly accelerated by a short period of ad libitum feeding before the wound is inflicted in CR animals [15]. In addition, accumulating data suggest that in mice housed in pathogen-free facilities severe CR increases susceptibility to infections by bacteria, virus and worms, even though CR has been shown to delay the age-dependent decline in certain immune functions [16].

Considering the breadth of organisms that respond positively to CR, should it be expected that non-human primates would likewise show similar results? There are two active randomized, non-human primates studies testing the benefits of long-term CR on disease prevention and longevity in rhesus monkeys, one at the University of Wisconsin at Madison and another at the National Institute on Aging (NIA) [17,18]. Both trials have shown that long-term, moderate (∼30%) CR can be safely initiated and maintained in a primate species. The recent results reported by the Wisconsin group are the first to show a significant CR-induced benefit in reducing age-related mortality and disease in rhesus monkeys [19]. However, when also deaths due to acute conditions (e.g., complications of anesthesia, gastric bloat, endometriosis, and injury) were included, overall mortality was not significantly different between the CR and control monkeys, even if the trend was still in the anticipated direction ($P = 0.16$). Although the demonstrated health and lifespan benefits are significant findings, a number of previous studies have suggested the plausibility of this outcome. Similar to rodents, CR in rhesus monkeys results in lower total and abdominal adiposity, improved insulin sensitivity and lipid/lipoprotein profile, decreased body temperature, decreased serum triiodothyronine concentration and reduced inflammation [20–23]. Despite the delay in knowing the final outcome of the full longevity study, the positive outcomes of the available data merit consideration. For example, CR resulted in a 50% reduction of age-related diseases, when considering cancer and cardiovascular disease [19]. The CR monkeys in the Wisconsin study were also fully protected against the development of obesity and glucose intolerance/type 2 diabetes [19]. Interestingly, even monkeys that had glucose metabolism impairment prior to initiation of the CR regimen, showed no impairment of glucose homeostasis years later [19]. Sarcopenia, a serious health concern associated with advancing aging, was also partially prevented in CR monkeys. In the Wisconsin study, body weight adjusted skeletal muscle mass declined more rapidly in the control than in the CR group [24]. Finally, CR monkeys showed improved T cell function and preservation of gray matter volume in several subcortical regions, including the caudate, putamen, left insula, and in others key regions related to motor function and aspects of executive function [19,25]. However, as rhesus monkeys have a maximum life span of 40 years, it may be another 10 years before maximal life span data become available on these primates.

3. Calorie restriction in humans

It is difficult to determine whether CR has beneficial effects on intrinsic aging and maximal lifespan in humans, because there are no validated biomarkers of aging and because it is impractical to conduct randomized, diet-controlled, long-term survival studies in normal-weight humans. Another potential problem is the inappropriate use of the term "calorie restriction" in clinical studies. In animal studies, CR refers to a state in which the energy intake is reduced by 30–50% below the levels consumed by a control group of animals that eats a chow diet "ad libitum", and not a high fat or sucrose diet that results in obesity. In addition, in some studies, food intake in the control group is limited (i.e., 85–95% of the calories of animals fed ad libitum) to avoid comparison of the CR group with control animals that gain some weight with age [26]. In contrast, in humans the term "CR" is often loosely used to describe any reduction in energy intake, even if the baseline energy intake is excessive (i.e., overweight/obese individuals) and it is being reduced to lower levels. We believe that this is misleading, because in the context of the aging/longevity studies the term "CR" should refer only to a state in which energy intake is sufficiently low to achieve or maintain a low-normal body weight status (i.e., body mass index <21 kg/m^2) without causing malnutrition (i.e., adequate intake of proteins and micronutrients). However, for the purposes of discussing CR in this review, we will focus on the metabolic and physiological effects of CR when applied to normal weight individuals, and will not discuss the role of CR in treating the pathological state of overweight/obesity.

Data from epidemiological studies suggest that CR has beneficial effects on human longevity. These studies include natural experiments, such as a study on the inhabitants of Okinawa (Japan) who were known to consume fewer calories than residents of the main Japanese islands [27]. Until 1960, the reported daily calorie intake of inhabitants of Okinawa Island was 1785 kcal/day, ∼15% and ∼40% less than the average calorie intake of a mainland Japanese (2068 kcal/day) and US (2980 kcal/day) resident, respectively [28]. In this older cohort of Okinawans (aged 65+) mortality from coronary heart disease and cancer was markedly lower than in the average mainland Japanese and US population [29]. As a consequence, Okinawa has approximately 50 centenarians per 100 000 inhabitants, one of the highest numbers of centenarians in the world [30]. Another category of studies in humans includes more controlled demonstrations of the effects of CR in normal-weight individuals, such as occurred with Biosphere 2 which took place in a closed ecosystem in Arizona from 1991 to 1993, involved four men and four women who experienced a forced decrease in calorie intake for 18 months, because of an unanticipated decrease in food availability [31]. During the first 6 months, the biospherians consumed ∼30% less calories (from ∼2500 to ∼1784 kcal/day), rising then to ∼2000 kcal/day for the remaining 12 months, while sustaining high levels of physical activity (∼70–80 h of -work/week) required by their daily duties. This combination of reduced energy intake and increased physical activity resulted in a reduction of many anthropometric and physiological parameters, including reductions in body weight, blood pressure, fast-

ing blood glucose, insulin, cholesterol, triiodothyronine and white blood cells [31].

Another series of metabolic and physiological studies have been conducted in members of the Calorie Restriction Society, which is a group that practices self-imposed CR in the belief that CR will extend their healthspan and lifespan. The CR group consists of lean volunteers, who had been eating about 1800 kcal/day for an average of 6.5 years, which is ~30% less calories than age-matched and sex-matched volunteers consuming a typical Western diet [32]. The CR society members eat a diet rich in nutrient-dense foods, including a wide variety of vegetables, fruits, whole grains, nuts, egg whites, fish, low-fat dairy products and lean meat, which supplies more than 100% of the recommended daily intake (RDI) for all essential nutrients. The decrease in energy intake resulted in a decrease in BMI from 23.7 kg/m^2 at the beginning of CR to a currently steady BMI of 19.6 kg/m^2 [32]; total body fat averaged 6.7% in the CR men and 22.4% in the comparison group men. The metabolic and physiological data from members of the calorie restriction society show that CR provides powerful protective effects against overweight/obesity, type 2 diabetes, inflammation, and left ventricular diastolic dysfunction that are similar to those that occur in CR rodents and monkeys [33,34]. Serum total cholesterol, low-density lipoprotein cholesterol, triglycerides, fasting glucose, fasting insulin were all significantly lower, whereas HDL-C was higher, in the CR group than in the US diet control group [34]. In particular, the CR society members appear to have much lower levels of blood pressure (both systolic and diastolic blood pressure) and inflammatory markers (i.e., C-reactive protein, tumor necrosis factor-α, and interleukin-6) than healthy, age- and sex-matched controls eating typical Western diets [33–35]. Based on a range of risk factors, it appears that long-term CR has a powerful protective effect against atherosclerosis and hypertension. This interpretation is supported by the finding of a low carotid artery intima media thickness, which was ~40% less in the CR group than in the comparison group [34]. Currently, the only known direct evidence that CR may influence intrinsic aging in humans is that CR society members who have been on CR for an average of 6.5 years have better left ventricular (LV) diastolic function than healthy age-matched and sex-matched controls [33]. Aging results in progressive increase in LV stiffness and impairment in diastolic function, that involves a slowing of LV relaxation, with a decrease in the rate of peak early, suction-mediated LV filling (E wave), whereas the relative contribution of the atrial component of LV filling (A wave) increases [36,37]. In the volunteers on CR, whose average age was 51 ± 12 years, the left ventricular diastolic function was similar to function in those who were approximately 16 years younger [33] and is consistent with the beneficial cardiac effects of CR observed in mice and rats [38].

Although research on CR in humans is still at an early stage, available information suggests that CR induces a number of the same adaptive response that occurs in laboratory animals. For example, CR results in some of the same hormonal adaptations related to longevity in CR rodents, including lower circulating concentrations of triiodothyronine, testosterone, and estradiol, and increased adiponectin and steroid hormone binding protein concentrations [35,39,40]. However, key differences in the metabolic effects of CR exist between mice and humans. In rodents, CR without protein restriction induces a 20–40% reduction in the level of insulin-like growth factor-1 (IGF-1), an important growth factor that mediates proliferation and inhibits apoptosis [41]. In contrast, in humans, severe CR does not reduce serum IGF-1 and IGF-1/IGFBP-3 concentrations, unless protein intake is also reduced [42]. In addition, ad libitum fed vegans consuming a mildly restricted protein diet (~0.75 g of protein/kg body weight/day; ~10% calories from protein) display significantly lower serum IGF-1 concentrations than CR individuals eating a relatively high protein diet (1.73 g of protein/kg/day; ~24% calories from protein) and sedentary individuals eating a typical Western diet (1.24 g of protein/kg/day; ~16% calories from protein), further suggesting that protein intake is more important than calorie intake in modulating circulating IGF-1 levels in humans [42]. This is important because the median protein requirement of the healthy adult population is 0.65 g/kg/day and the recommended daily allowance (covering the entire population) is 0.83 g of protein/kg of body weight/day [43]. This is close to the average protein intake of individuals eating a vegan diet. In contrast, in many developed and developing countries people are eating $\geqslant$1.2 g of protein/kg of body weight/day, that is $\geqslant$30% protein than the RDA recommended intake [44], which is presently considered to be harmless or even beneficial against the development of obesity, sarcopenia, osteoporosis. However, data supporting a protective role of high-protein diets against these diseases are limited and controversial, whereas there is considerable evidence that a reduction in IGF-1 or IGF-1 signaling plays a key role in modulating cancer and aging in rodents and humans [45–48]. More studies are urgently needed to understand the metabolic and clinical implications of consuming high protein diets on serum IGF-1 and IGFBPs concentrations, and on cancer biology, especially in sedentary adults with a positive family history for prostate, breast (pre-menopausal) and colon cancer.

4. Metabolic and molecular mechanism of CR

Since 1935, many mechanisms have been proposed as the biological basis of the life-prolonging and anti-aging actions of CR; none is strongly supported by available evidence, but it is entirely possible that the actions of CR involve a combination of metabolic, physiological and cellular adaptations to CR itself [49,50]. It is well established that nutrient-sensing pathways are key modulators of the aging process; different nutrients can activate different pathways directly or indirectly [7]. For example in mice, CR down-regulates the insulin/IGF-1/mTOR pathways, which, in turn, activates other anti-aging pathways in various mammalian cells [51,52]. Mutations that cause a down-regulation of the insulin/Igf-1/mTOR signaling pathways can substantially increase healthspan and lifespan in mice [7,53]. For example, Ames dwarf mice that carry a loss-of-function mutation in the gene Prop1^{df} that leads to abnormally low expression of GH, TSH and prolactin [54], live >50% longer than their normal siblings [55]. Growth hormone (GH)-deficient and GH receptor-deficient mice, which have also low circulating IGF-1 levels, live substantially longer than wild type mice. GHR-BP knockout and GH-deficient mice have lower incidence and delayed occurrence of tumors, increased insulin sensitivity, and a reduction in age-dependent cognitive-impairment [53,56–58]. In addition, decreased IGF-1 signaling is involved in the delayed aging phenotype of IGF-1 receptor-deficient mice, klotho transgenic mice, and pregnancy-associated plasma protein A (PAPP-A) knock-out mice [59-61]. In contrast, mice overexpressing the GH receptor have very high concentrations of IGF-1, larger body size, shorter lifespan, and an increased incidence of cancer, kidney and neurodegenerative disease [62]. In addition to alteration in IGF-1 signaling, alterations of the insulin and mTOR pathways appear to contribute to the effect of CR on longevity. For example, loss-of-function mutations in the insulin receptor in adipose tissue, and in insulin receptor substrates 1, and 2 in the brain all promote longevity in mice [63–65]. Inhibition of the mTOR pathway by genetic deletion of the ribosomal S6 protein kinase 1 (S6K1) increases maximal lifespan of female mice only, and reduces the incidence of several age-associated disease [66]. In addition, supplementation with rapamycin (a drug that inhibits mTOR), but not resveratrol or simvastatin, sig-

nificantly increases maximum lifespan of both female and male mice [67,68].

Other important CR-mediated neuroendocrine adaptations, that have been hypothesized to play an important role in mediating the anti-aging effects of CR, are: (1) reduced levels of hormones that regulate thermogenesis and cellular metabolism (e.g., thyroid hormones, cathecolamines), (2) reduced levels of anabolic hormones (e.g., testosterone, estradiol, insulin, leptin), and (3) increased levels of hormones that suppress inflammation (e.g., glucocorticoids, adiponectin, ghrelin) [69]. For example, it has been shown that maximal lifespan can also be extended by mutations of genes encoding proteins along pathways regulating hormonal and mitogenic signals (e.g., p66shc, type 5 adenylyl cyclase, angiotensin II type 1 receptor) [70–72]. In addition, ad libitum-fed transgenic mice overexpressing the uncoupling protein 2 in hypocretin neurons (Hcrt-UCP2) have lower core body temperature, and a 16% greater life expectancy than wild type animals, independently of caloric intake [73].

Accretion of oxidative damage with time has been hypothesized to play a central role in the biology of aging and age-associated diseases (Harman theory of aging) [74]. Oxidative damage to macromolecules (i.e., DNA/RNA, proteins and lipids) in cells and tissues exponentially increases with aging. Long-term CR reduces the age-associated accumulation of oxidative damage to proteins, lipids and DNA [75]. This attenuation of the accumulation of oxidative damage can be due to either a decreased rate of generation of reactive oxygen molecules, or to increased efficiency of protective processes, or to an increase in repair activity, or to a combination of these processes. However, most of the evidence in support of the Harman theory of aging is just associative, and accumulating data do not support a key and independent role of oxidative stress in modulating aging in mammals [76]. Indeed, supplementation with several combinations of antioxidants does not increase lifespan in laboratory rodents [77–79]. In humans, several sizeable long-term randomized clinical trials of supplementation with antioxidant vitamins have shown no reduction in cardiovascular or cancer morbidity/mortality [79-,81]. Moreover, rodents with genetic deletion of several antioxidant enzymes (e.g., Sod2+/−, Prxd1+/−, and Sod1+/− mice) do not have a shorter lifespan, despite having elevated oxidative stress markers and cancer incidence [82,83]. Overexpression of major antioxidant enzymes (i.e., CuZnSOD, Mn superoxide dismutase, and catalase overexpression; combinations of CuZnSOD and catalase or CuZnSOD and MnSOD overexpression), which are known to scavenge cytosolic and mitochondrial superoxide and hydrogen peroxide, does not extend lifespan or reduce the incidence of age-related disease in these mice [84,85]. The only experimental study showing an anti-aging role of endogenous antioxidant enzymes was done by the Rabinovitch's group. This study showed that transgenic mice overexpressing catalase targeted to mitochondria increased maximal lifespan, suggesting that increased antioxidant defense system in the mitochondrial compartment may be involved in promoting longevity [86]. Thus, whether CR's ability to reduce oxidative damage plays a major role in its life-extending action, still remains an open question. It is possible that the reduction in oxidative damage in CR mice, p66shc knockout, klotho transgenic mice and IGF-1 signaling deficient mice is just an epiphenomenon rather than the causal link.

Another mechanism that has been proposed to play a role in mediating some of the anti-aging effects of chronic CR is hormesis, referring to the phenomenon whereby, a usually detrimental environmental agent (e.g., radiation, chemical substance) changes its role to provide beneficial effects when administered at low intensities or concentrations. CR has been hypothesized to be a low-intensity stressor that provokes a survival response in the organism, helping it to tolerate adversity by activating longevity pathways [87]. Indeed CR leads to a modest increase in the daily peak concentration of plasma free corticosterone in rats and mice; this chronic increase would be expected to have a significant anti-inflammatory and anti-cancer action [88]. At the cellular and molecular level CR may induce an increase in the activity of genes that protect cells from the damaging action of harmful agents. Indeed, CR has been shown to increase the induction of hepatic Hsp70, one of these scavenging proteins, in response to heat stress [89]. Moreover, CR has been shown to enhance autophagy and DNA repair systems, and up-regulate endogenous enzymatic and non-enzymatic antioxidative defense mechanisms [90–92].

5. Conclusions

The prevention of age-associated chronic disease and the promotion of healthy aging are key issues in the challenge to improve health, delay the onset of frailty and dependency, and reduce healthcare costs. Data from epidemiological and clinical studies show that many metabolic alterations and age-associated illnesses can be prevented or reversed, with the implementation of healthy lifestyle interventions [93–95]. Data are accumulating on the effects of CR in non-human and human primates. We know that in both non-human and human primates CR without malnutrition results in many of the same metabolic, hormonal and physiological adaptations related to longevity in CR rodents. We also know that in both monkeys and humans, CR with adequate nutrition, protects against abdominal obesity, type 2 diabetes, and cardiovascular diseases, which are leading causes of morbidity and mortality. Cancer incidence and mortality are also reduced in CR monkeys, and studies of CR humans show a reduction of a series of metabolic and hormonal factors associated with increased cancer risk. Moreover, a moderate restriction of protein intake may have additional beneficial effects in preventing cancer. Nonetheless, nothing is known about the long-term effects of CR on wound healing, on the risk of developing infections and cognitive impairment, and on the rate of aging in non-human and human primates. More studies are needed to elucidate the molecular mechanisms underlying the beneficial effects of CR in non-human and human primates, so that we can develop new markers/targets of aging/longevity.

Acknowledgments

Support was provided by the National Center for Research Resources (grant UL1 RR024992) and the National Institute of Diabetes and Digestive and Kidney Diseases (grant P30DK056341); by grants from Istituto Superiore di Sanità/National Institutes of Health Collaboration Program, Ministero della Salute, the Longer Life Foundation (an RGA/Washington University Partnership), and the Bakewell Foundation; and by a donation from the Scott and Annie Appleby Charitable Trust. The funding agencies had no role in the analysis or interpretation of the data or in the decision to submit the report for publication. The authors had no conflicts of interest.

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