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Caloric Restriction to Moderate Senescence: Mechanisms and Clinical Utility

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Abstract

As life expectancy in the United States continues to increase, the maintenance of physical independence among older Americans has emerged as a major clinical and public health priority. Therefore, there is an urgent need to identify interventions that can maintain or enhance cognitive and physical function with the goal of preventing or delaying the onset of disability. To date, caloric restriction (CR) is the only method that has been consistently found to increase lifespan and delay the onset of age-associated diseases such as cancer and diabetes across multiple species. The promise of calorie restriction as an intervention to improve health and/or maintain function in humans, however, only holds if individuals are able to adhere to this intervention over the long-term. Unfortunately, long-term adherence to CR regimens is notoriously poor likely due to complex interactions between behavioral, physiological, psychological, and environmental variables. Thus, a current challenge for both researchers and clinicians is to identify methods that can assist individuals in maintaining CR over the long-term.

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Conflict of Interest

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Keywords

Caloric restriction; Aging; adherence; lifestyle; health-span; life-span; body weight; appetite

Introduction

Aging is associated with a host of biological changes that contribute to a progressive decline in cognitive and physical function, ultimately leading to a loss of independence and increased risk of mortality. A number of factors have been associated with the pathogenesis of functional decline during aging and physical disability (in non-acute disease conditions), but the exact mechanisms contributing to this process remain largely undefined. As life expectancy in the United States continues to increase, the maintenance of physical independence among older Americans has emerged as a major clinical and public health priority [1]. Therefore, there is an urgent need to identify interventions that can maintain or enhance cognitive and physical function with the goal of preventing or delaying the onset of disability [2].

To date, caloric restriction (CR) is the only method that has been consistently found to increase lifespan and delay the onset of age-associated diseases such as cancer and diabetes across multiple species including nonhuman primates [3]. Findings from animal studies suggest prolonged CR has the potential to extend health-span, the period of life when an individual is functional and free of serious illness, and potentially lifespan [4]. The precise mechanism through which CR exerts these beneficial effects is not known; however, findings from basic science indicate that CR produces broad, systemic effects that can directly influence health and functional ability and thereby extend health-span. The beneficial effects of CR appear to be conserved across various animal species [5], and short-term studies demonstrate that the basic physiology of caloric restriction is well conserved in humans [6]. Moreover, findings from initial short-term randomized clinical trials support the promise of CR to improve health and potentially extend health-span and longevity in humans. Thus, there is hope that the beneficial effects of CR on delaying the onset of age-related disease conditions observed in various nonhuman species, including in nonhuman primates, can be translated to humans.

The promise of CR as an intervention to improve health and/or improve function in humans, however, only holds if individuals are able to adhere to this intervention over the long-term. A concern associated with calorie-restricted diets is that they do not lead to lasting weight loss or health benefits and may lead to loss of fat-free mass adverse changes in body composition over time [7]. Therefore, a current challenge is to find ways minimize the loss of fat-free mass that typically occurs following CR, while assisting individuals in maintaining CR over the long-term. In the sections below, we review the following: (1) definition of CR, (2) basic mechanisms and physiological factors through which CR may exert its beneficial effects, (3) findings from animal and non-human primate studies, (4) findings from initial clinical trials in humans, (5) translation of CR to non-overweight individuals, (6) long-term utility of CR, (7) strategies to enhance adherence to CR, and (8) alternative dietary approaches that may produce similar benefits of CR.

1. Definition of Caloric Restriction

Currently, there is no precise or universally agreed upon definition of CR. Some researchers have suggested that CR should be defined as a reduction in calories of approximately 30% from eating freely until satiation [8]. More recently, experts have suggested that the beneficial effects of CR begin at approximately 25–30% reduction from free living food intake and remain present up to a 50–65% reduction in food intake (Watt et al., 2013). These

recommendations are based on initial studies in rodents which demonstrated that mice fed 55–65% caloric restricted diets through their life exhibited a 35–65% greater mean and maximal lifespan compared to mice eating a non-purified ad libitum diet [9]. Notably, reductions in food intake greater than this amount for prolonged periods lead to a host of negative effects [10], as it is likely CR transitions into starvation at this point.

A limitation of many pre-clinical studies that have examined the effects of CR is that the animals in the comparison group are typically fed an ad libitum diet, which is known to lead to excessive food consumption and weight gain. Thus, the dietary intake of the animals in the ad libitum group would not be reflective of a "healthy" eating pattern. Additionally, animals are frequently confined within a limited space, which is likely to reduce physical activity levels. This combination of environmental factors (i.e., readily available food and limited space for movement) is likely to contribute to unhealthy weight gain and ultimately increase risk of disease conditions that are known to be associated with obesity and sedentary lifestyle. A more strict comparison group would be one in which food is provided at a level sufficient to maintain a healthy body weight and produce weight stability. Fortunately, this type of comparison group has been used in recent randomized controlled trials testing the effects of CR in overweight humans. The findings of these trials are described in Section 4, following a brief discussion of the basic mechanisms through which CR exerts its effects and description of findings from animal models.

2. Basic Mechanisms

The beneficial effects of CR during aging are thought to be due, in large part, to its powerful anti-oxidative action [5] and ability to maintain a proper cellular redox status as evidenced by suppressed oxidative damage to lipids, DNA, and proteins [11;12]. Oxidative damage appears to be closely linked with systemic inflammation, and recent research suggests chronic molecular inflammation is a major biological mechanism underpinning the aging process and age-related diseases [5]. Recent evidence has documented CR's anti-inflammatory action, as shown by its modulation of pro-inflammatory genes (e.g., TNF-α, IL-1, IL-6) and adhesion molecules (e.g., VCAM-1, E-selectin) through the NF-κB signaling pathway [5].

In addition to promoting proper redox status and its anti-inflammatory action, CR may confer significant health benefits through additional biological processes that can enhance cellular quality control including autophagy (i.e., "self-eating" of damaged organelles), activation of the ubiquitin-proteosome system (UPS; removes damaged proteins), and the maintenance of a healthy population of mitochondria though biogenesis (i.e., generation of new mitochondria) [13;14]. Autophagy is an evolutionary conserved process that allows eukaryotic cells to degrade and recycle long-lived proteins and organelles [15]. The decline in autophagic and proteasomal activity observed during aging has been proposed to contribute to different aspects of age-related phenotypes, such as neurodegeneration, osteoarthritis, declining liver and T-cell function, as well as age-related muscle loss [16;17]. Caloric restriction increases the activity and effectiveness of autophagy and the UPS [18;19]. Additionally, CR can support healthy population of mitochondria through increasing mitochondria biogenesis (Civitarese *et al*. 2007; Dutta *et al*. 2012), as well as oppose the reductions in mitochondrial function that occur during aging by affecting the expression of genes such as PGC-1α [20].

3. Findings from Animal and Non-human Primate Studies

The measurement of the effect of CR on longevity parameters has been studied across a variety of species over nearly the past century [4;21–24]. Caloric restriction has now been shown to increase longevity in a variety of species including rats [24], mice [25], yeast [26–

30], flies [31–33], water fleas [34], nematodes [35–38], rotifers [39;40], spiders [41], fish [42], hamsters [43], and dogs [44;45]. The range of lifespan extension is typically between 30–60% in rodents [4;25], but can vary significantly across species. In addition to extending lifespan, prolonged CR in rodents has also been found to delay the onset of age-associated disease conditions such as cancer and diabetes [24;46]. Overall, findings from animal studies suggest prolonged CR has the potential to extend both health-span and lifespan, and improve quality of life during extended years.

In comparison to studies in short-lived species, the study of the effects of CR on health and aging parameters in non-human primates is relatively novel. Two main agencies have been involved in this line of research, the National Institute on Aging (NIA) and the Wisconsin National Primate Research Center (WNPRC), and studies focus on the effects of CR ≤ 30% in rhesus monkeys. Since 1987, the NIA study has been examining male and female monkeys introduced to CR at different stages throughout their lifespan. Control monkeys, kept on a highly nutritious control diet, received two meals per day sufficient to attain apparent satiety, whereas the CR group received 30% fewer calories, adjusted for age and body weight [47]. In 1989, the WNPRC began to study both male and female monkeys that were introduced to 30% CR as adults [47].

Findings related to mortality and morbidity are not conclusive at present, but initial findings from the WNPRC study indicate that moderate CR is effective in lowering the incidence of aging-related deaths in adult rhesus monkeys [3]. At the time of the data analysis for this publication, the majority of the CR monkeys had survived compared to only half (50%) of control fed monkeys. Later findings by Mattison and colleagues (2012) indicate that the CR regimen implemented in rhesus monkeys at the NIA did not improve survival outcomes [48]. These findings suggest the effects of CR on markers of health, which were consistently found in both the NIA and WNPRC studies, may not translate to reductions in morbidity and mortality. Consequently, additional evidence is needed to better understand the effects of CR on mortality and morbidity in nonhuman primates.

Preliminary and interim data analyses in both the NIA and WNPRC studies indicate that CR has a number of positive effects on a wide variety of health parameters including body weight, glucoregulatory function, risk for cardiovascular and diabetes, muscle loss, incidence of neoplasia, bone health, and brain morphology [3;47;49;50]. The post-maturational decline in DHEAS was significantly attenuated in adult CR monkeys compared to controls after three to six years of CR [51]. Additionally, melatonin levels in old CR monkeys (20–35 years old) were significantly greater than those observed in age-matched controls [52]. In the NIA study, pentosidine and furosine (i.e., two glycation products known to increase with age) showed positive correlations with age in CON monkeys but not in the CR monkeys [47].

4. Translation to Humans

The effect of CR in humans has been examined in randomized controlled trials; however, results are limited to overweight adults. The Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy (CALERIE) was a multicenter study testing the hypothesis that CR improves biomarkers of longevity [53]. During Phase 1, independent studies were conducted at three different sites: Pennington Biomedical Research Center, Jean Mayer, USDA Human Nutrition Research Center on Aging (HNRCA) at Tufts University, and the Washington University School of Medicine. An important goal of Phase 1 was to provide critical information to inform about appropriate study design to use in longer-term trial during Phase 2.

At the Pennington Biomedical Research Center, overweight (25 ≤ Body Mass Index [BMI] < 30 kg/m^2) but otherwise healthy adults (25 to 50 years old) participated in 24 weeks of CR. Participants were randomized to one of four groups: (1) Healthy Weight Control; energy intake matched to energy requirements; (2) CR; 25% diet restriction; (3) CR plus exercise group (CREX); 12.5% CR plus a 12.5% increase in energy expenditure; and (4) low calorie diet group (LCD); 890 kcal/d diet until they reached 15% reduction in body weight followed by maintenance. Participants in all groups, except the LCD group, were provided with all meals during baseline and for 12 weeks following randomization, and then were asked to self-select their food based on individually designed calorie targets. During weeks 22–24, they resumed the in-feeding diet. All diets (except the LCD) were based on recommendations per the American Heart Association (30% fat; [54] and participants in the LCD group consumed five shakes per day equaling 890 kcal/d. Participants attended weekly group meetings and reported energy intake weekly by telephone. Skills to modify eating behavior and increase adherence to diet and exercise were taught using a cognitive-behavioral approach during group meetings. Participants in the CREX group engaged in a supervised, structured exercise program on three days per week. In addition, participants completed exercise on two additional days and used a heart rate monitor to track their efforts. During Baseline, Month 3, and Month 6 visits, participants attended a 5-day inpatient stay where numerous metabolic tests were conducted [53].

The key findings from Phase 1 of the CALERIE trial conducted at the Pennington Biomedical Research Center included the following measures: (1) percent weight change was significantly reduced among all participants, (2) All participants in diet groups had reductions in 24-h energy expenditure and DNA damage, (3) two biomarkers of longevity (fasting insulin and body temperature) were significantly reduced from baseline in the CR and CREX groups, and (4) dehydroepiandrosterone (DHEAS) and glucose were unchanged from baseline across all groups [53]. Participants in the CR and CREX groups lost approximately 10% of their initial body weight, significantly reduced triacylglycerol, and increased HDL-cholesterol; thus their estimated 10-year CVD risk [55] significantly declined from baseline by 29% in the CR group and 38% in the CREX group while remaining unchanged in the control group [56]. There was a significant decline in both fat and fat-free mass in both the CR and CREX groups. Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were also significantly reduced from baseline in both CR and CREX groups, with no adverse changes in bone mineral density for total body or hip measures [57;58]. A significant increase in mean growth hormone (GH) and total insulin-like growth factor-1 concentrations in the CREX and LCD groups [59], and a significant decrease in liver lipid content in the CR and LCD groups [60]. Moreover, participants in the CREX group showed a significant decrease in heart rate and sympathetic nervous system index, while demonstrating an increase in parasympathetic nervous system activity [61]. After six months of CR, mitochondrial DNA content increased by 35% in the CR group and 21% in the CREX group, but did not change in the control group, and DNA damage was reduced in both CR and CREX groups, but not in the control group [62].

At the Jean Mayer, USDA Human Nutrition Research Center on Aging (HNRCA) at Tufts University, overweight (25 ≤ BMI < 30kg/m^2) but otherwise healthy adults (24–42 years old) participated in one year of CR. Participants were randomized to either 30% CR or control group. Within each group, participants were also randomized to either a high glycemic load diet (i.e., mean estimated daily glycemic index of 86; HG) or low glycemic load diet (i.e., mean estimated daily glycemic index of 52; LG) based on the International Tables of Glycemic Index and Glycemic Load [63]. During the first phase (24 weeks) of the intervention, all food was prepared according to CR prescription and provided to the participants. During the second phase (24 weeks) of the intervention, participants were

responsible for their own food preparation. To maximize adherence, participants attended weekly behavioral support groups and individual meetings with the study dietitian [64].

The key findings from Phase 1 of the CALERIE trial conducted at Tufts University revealed that participants in both intervention groups had significant weight loss, decrease in mean body fat percentage, and decrease in resting metabolic rate (RMR) from baseline [64]. Levels of triacylglycerol, insulin, as well as total, High-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol decreased over time [64]. Participants with high insulin secretion at baseline lost more weight if randomized to the LG diet compared with the HG diet, compared to participants with low insulin secretion at baseline in the HG diet who lost more weight than those in the LG diet, but the difference was not statistically significant [65]. After six months of CR, activity of glutathione peroxidase (GPx) was increased, and markers of oxidative stress (i.e., plasma protein carbonyl and 8-Hydroxydeoxyguanosine) decreased. Changes in BMI, percent body fat, and fat mass were not found to be associated with changes in any of the markers of oxidative stress [66].

At the Washington University School of Medicine CALERIE site, healthy, non-obese (BMI range = 23.5–29.9 kg/m^2) men and postmenopausal women (50–60 years old) were randomly assigned to one of three groups: (1) the CR group; (2) the Exercise (EX) group; or (3) the Healthy Lifestyle (HL) group (control). The goal of the CR group was to gradually increase caloric restriction from 16% during the first three months to 20% during the remaining nine months of the intervention. Meals were provided for five consecutive days during both the 16% CR and 20% CR phases to help participants adjust to their prescription. Participants in the CR group attended weekly individual and/or group meetings for the first six months, then less frequently throughout the intervention. The goal of EX groups was to increase energy expenditure through exercise without changing caloric intake. Participants in the EX group gradually increased their energy expenditure from 16% to 20% and the intensity and frequency of exercise was personalized for each participant. Adherence was monitored by heart rate monitors and weekly weigh-in sessions throughout the intervention [67]. Based on doubly-labeled water measurement, participants achieved a mean CR of 11.5 ± 2.1% after one year based. Examination of food diaries revealed that energy intake decreased significantly in the first six months, but participants were less adherent during the final six months [67].

The key findings from Phase 1 of the CALERIE trial conducted at the Washington University School of Medicine for the CR and EX groups include (1) significant weight loss, (2) significant reductions in whole-body fat mass, (3) significant reductions in lean mass, and (4) significant decreases in VAT and SAT [68]. Additional analyses revealed a significant decrease in fasting insulin and insulin AUC, as well as an increase in insulin sensitivity index in the EX and CR groups, but not in the HL group. Serum adiponectin concentration tended to increase in the EX and CR groups and decreased significantly in the HL group; however, there were no differences between groups in fasting glucose or adjusted serum TNFα concentrations [69]. Both interventions significantly reduced oxidative damage to DNA and RNA [11]. Overall, reductions in most of the major coronary heart disease risk factors, including plasma LDL-cholesterol, total cholesterol/HDL ratio, and C-reactive protein concentrations were similar in both the CR and EX groups [70]. Physical fitness was improved in the EX group only as demonstrated by an increase in VO2max, compared to a decrease in the CR group [68].

5. Translation to Non-Overweight Individuals

A key issue for future research is whether or not the promising findings from initial CR trials in humans translate to humans who have a body mass index in the healthy range (i.e., BMI range = 20.0–24.9 kg/m^2). This question is currently being examined in Phase 2 of the

CALERIE study, which utilized a multicenter, single-protocol focused on achieving and maintaining 25% calorie restriction in healthy, non-obese ($22.0 \leq BMI < 28.0$ kg/m^2) middle-aged men and women (21–50 years old) throughout a 2-year intervention period [71]. Specifically, Phase 2 of the CALERIE study is examining whether a 25% reduction in energy intake improves markers of aging, cardiovascular disease risk, insulin sensitivity and secretion, immune function, neuroendocrine function, quality of life, and cognitive function as compared to ad libitum intake. The results from this study will have important implications for our understanding of the impact of calorie restriction on biological changes associated with aging and age-related disease conditions [71].

6. Long-Term Utility

The promise of CR suggested by findings from the CALERIE study and other clinical trials only holds if individuals are able to maintain CR over the long-term, as benefits are unlikely to remain present once individuals stop engaging in CR. Unfortunately, long-term compliance to dietary restriction regimens used in conventional weight loss programs is notoriously poor [72;73]. Two important biological compensatory mechanisms that increase the difficulty of sustaining CR are neuroendocrine signals and metabolic adaptation. During CR, anabolic output from neural pathways increases food intake while simultaneously suppressing energy expenditure. These neuroendocrine signals that occur during calorie restriction and following weight loss persist with prolonged weight reduction [74;75]. In addition, the reduction in total energy expenditure following weight loss is often greater than would be anticipated by the loss in lean body mass; a phenomenon termed metabolic adaptation. This phenomenon can lead to weight loss outcomes that are less than would be predicted by standard models [76].

Another major factor contributing to the difficulty of maintaining CR over the long-term is our “toxic environment,” where tasty high-fat, high-calorie foods are highly visible and easily available [77]. Exposure to an environment rich in palatable and calorically-dense foods can make sustained CR very challenging [77–79]. Complex interactions of physiological, psychosocial, and environmental factors can also increase the difficulty of maintaining CR over the long-term. For example, following a period of restrictive dieting, people often experience a greater sensitivity to palatable food [80;81].

7. Strategies to Promote Long-Term Adherence to CR

A variety of behavioral approaches have been tested to promote long-term adherence to reduced calorie diets in both the CALERIE trial, as well previous weight loss interventions. In particular, long-term adherence has been found to be improved when participants receive continued contact with counselors and/or other forms of positive social support [82]. Additionally, specific behavior skills including goal setting, self-monitoring, stimulus control, and problem solving have consistently been found to facilitate short- and long-term adherence to reduced calorie diets [82]. Specific dietary strategies, such as consumption of low energy diets, have also been found to increase satiety levels and may be useful in promoting adherence to CR regiments [83]. The clinical utility of caloric restriction will likely be enhanced through continued testing of behavioral and dietary approaches to facilitate behavior change. Moreover, identification of pharmacological targets in the brain that may directly impact appetite control is needed. Thus, further study of the role that botanical compounds may have in facilitating appetite control is warranted in human clinical trials.

8. Alternative Dietary Approaches

An important area in need of further investigation is whether alternative dietary approaches, such as intermittent fasting, can produce biological changes and reductions in disease risk

similar to caloric restriction [84–86]. Findings from initial clinical trials suggest that this type of dietary approach has significant potential to improve markers of health, in a manner that is independent of weight loss. A potential limitation of this type of dietary approach, however, is that it could lead to large quantities of food being consumed during non-fasting periods, which has the potential to produce adverse metabolic effects [87;87;88]. Further research is needed to explore the potential risks/benefits associated with this type of eating pattern.

Conclusions

Caloric restriction has consistently been shown to delay the onset of age-related diseases and extend lifespan in numerous species including nonhuman primates. Findings from initial clinical trials in humans support the promise of CR suggested by pre-clinical studies to reduce disease risk and extend health-span. Unfortunately, long-term compliance to CR is notoriously poor and remains problematic. Although the specific reasons that long-term compliance to CR regimens is so difficult are not yet known, complex interaction between physiological, psychological, behavioral, and environmental variables likely contribute to the challenge of maintaining CR over the long-term. In response to diet-induced weight loss, internal feedback systems signal the body to increase food intake and decrease energy expenditure. These signals do not decrease over time and likely increase the difficulty of maintaining CR in an environment filled with readily available, highly palatable foods. Moreover, food-related sensory experiences, particularly sensitivity to palatable foods, appear to be enhanced following extended periods of CR, which likely further contributes to the difficulty in maintaining CR over the long-term. Given the multiple challenges that most individuals experience in sustaining CR, research is needed to further explore novel behavioral, dietary, and biological interventions that can assist individuals in adhering to CR regimens. Additional areas of research that deserve attention is whether alternative dietary approaches, such as intermittent fasting, can produce similar beneficial effects as CR, as well as whether this type of eating pattern may be maintained over the long-term.

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