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Calorie Restriction: What Recent Results Suggest for the Future of Aging Research

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Abstract

Background—Calorie Restriction (CR) research has expanded rapidly over the past few decades and CR remains the most highly reproducible, environmental intervention to improve health and extend lifespan in animal studies. Although many model organisms have consistently demonstrated positive responses to CR, it remains to be shown whether CR will extend lifespan in humans. Additionally, the current environment of excess caloric consumption and high incidence of overweight/obesity illustrate the improbable nature of the long-term adoption of a CR lifestyle by a significant proportion of the human population. Thus, the search for substances that can reproduce the beneficial physiologic responses of CR without a requisite calorie intake reduction, termed CR mimetics (CRMs), has gained momentum.

Material & Methods—Recent articles describing health and lifespan results of CR in nonhuman primates and short-term human studies are discussed. Additional consideration is given to the rapidly expanding search for CRMs.

Results—The first results from a long-term, randomized, controlled CR study in nonhuman primates showing statistically significant benefits on longevity have now been reported. Additionally, positive results from short-term, randomized, controlled CR studies in humans are suggestive of potential health and longevity gains, while test of proposed CRMs (including rapamycin, resveratrol, 2-deoxyglucose and metformin) have shown both positive and mixed results in rodents.

Conclusion—Whether current positive results will translate into longevity gains for humans remains an open question. However, the apparent health benefits that have been observed with CR suggest that regardless of longevity gains, the promotion of healthy aging and disease prevention may be attainable.

Keywords

Calorie Restriction; Dietary Restriction; Aging; Longevity; Lifespan; Mortality

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Introduction

Research interest in aging and age-related disease progression has rapidly increased during the last half century. Particularly over the last 2 decades, model organisms including yeast, worms, flies and mice have produced a wealth of information demonstrating an interaction between genes and environment in determining longevity. One particularly active area of research has been the influence of diet on longevity and age-related disease. In this field, calorie (energy) restriction (CR), sometimes referred to as dietary restriction (DR), has been repeatedly shown to significantly increase lifespan and reduce age-related disease compared with *ad libitum* (*AL*) feeding conditions. Other works report much of the background and historical context for the benefits observed with CR [1;2]. The focus of this article is the expected results of CR in primate models, including human outcomes, as well as the potential of alternatives to CR, particularly the rapidly growing area of calorie restriction mimetic (CRM) research, to improve health and delay death.

Should CR be expected to produce health and longevity benefits in nonhuman primates?

Most CR research on longevity in mammals has been performed in rodents, with laboratory mice, *Mus musculus*, predominant during recent history [1–3]. However, it should be noted that a host of other organisms have shown similar benefits including yeast, nematodes, flies, rotifers, spiders, fish, rats, hamsters and dogs [1–4]. Considering the breadth of organisms that respond positively to CR, should it be expected that nonhuman primates would likewise show similar results? There are two active randomized, nonhuman primate studies testing the benefits of long-term CR on longevity and disease in rhesus monkeys, *Macaca mulatta* – one at the University of Wisconsin at Madison and another at the National Institute on Aging (NIA) [5–7]. A third, non-randomized study at the University of Maryland with a smaller number of restricted monkeys on a weight maintenance diet has interpreted results in the context of CR as well. [8–11]. The two randomized studies were begun approximately two decades ago, such that results currently being reported benefit from the forethought of multiple researchers [5;7].

The recent results reported by Colman et al. (2009) are the first results from the Wisconsin CR study showing a significant benefit in reducing age-related mortality and disease with CR in rhesus monkeys [12]. When measuring mortality in a longevity study, consideration should be given to the cause of death, when possible. This can be exemplified by a study subject who dies in an accident (e.g. an automobile collision), which results in a mortality event, but not necessarily a result of the experimental treatment or aging process. In a similar way, even in a well-controlled longevity study, animals can encounter “accidents” which result in mortality, potentially independent of their underlying biological aging process. Thus, after censoring monkeys for what were considered non-age-related mortality events, like gastric bloat, anesthesia complications, endometriosis and injury (7 control and 9 CR monkeys), a significant lifespan benefit with CR (P=0.03 [Cox Regression Analysis]) was observed (age-related mortality events/group: Control:n=14, CR:n=5) [12]. However, when assessing “all-cause” mortality in all monkeys in the study and considering the interim mortality results for each group (all cause mortality events/group: n=21/38 control and 14/38 CR), CR does not currently provide a statistically significant lifespan increase (P=0.16 [Cox Regression]), although there is a difference in the expected direction [12]. The significance of the lifespan benefit observed on “age-related” mortality with CR is noteworthy, considering the reduced power of this analysis due to the relatively small sample size [12].

Although the demonstrated health and lifespan benefits are significant findings, a number of previously published interim reports and other studies have suggested the plausibility of this

outcome. Similar to rodents, CR in rhesus monkeys results in reduced circulating glucose and improved insulin function, decreased core body temperature, decreased body weight and fat, improved blood lipids and maintenance of dehydroepiandrosterone levels [8;9;13–23]. 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 significant reduction of age-related diseases, when considering neoplasias, glucoregulatory impairment and cardiovascular disease (respective incidence controls: 8, 16, 4 vs. CR: 4, 0, 2) [12]. This reduction of age-related disease and a potential increase in longevity are promising, although the results on total mortality are not yet definitive with CR [12]. Moreover, results demonstrating a significant benefit on longevity in the NIA's CR monkey study are not yet available [24;25]. However, available data point to a reduction in disease risk and incidence. Based on these prospects, if CR does indeed improve health and potentially increase lifespan in monkeys, will it do so in humans?

Even if CR works in monkeys, will CR work in humans?

Extensive knowledge exists about human responses to energy restriction or CR. However, with a few notable exceptions [26–28], the supporting data are largely derived from the implementation of a dietary reduction to cause weight loss among overweight or obese persons. As informative as this may be, the ultimate question of whether CR will extend longevity and slow age-related disease in humans cannot be answered in the context of pre-existent obesity. By CR, one could simply be returning an unhealthy, disease-promoting state back to the norm without altering the underlying aging process [29]. Others have proposed this may be the case in rodent CR studies [30–33] and could potentially influence the interpretation of the non-human primate studies as well. Although this may be a potential confounder in laboratory studies of CR, it also has implications for the majority of the human population in the developed world, particularly with the rise in overweight/obesity prevalence in modern times [34]. Nevertheless, the ultimate question of whether CR alters aging and disease in otherwise healthy individuals has much less data available. One argument proposes that CR is not a universal phenomenon, and in combination with the variability of the response, the life history theory of longevity suggests there is no reason to believe that the relatively small reproductive costs of humans will result in a favorable tradeoff of lifespan extension [29;35;36]. On the other hand, because of the breadth of organisms that respond favorably to CR, the potentially conserved molecular and cellular mechanisms, and the evidence from the nonhuman primate and short- to medium-term human studies, it is reasonable to expect that the observed health benefits would translate into longevity gains [37–39].

To better address this question, the NIA has recently funded a multi-site human randomized clinical research study to assess the effects of two years of CR (~25% restriction) in non-obese, healthy individuals through the CALERIE study (Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy) (http://calerie.dcri.duke.edu/index.html). Preliminary results from these human studies are reproducing many of the metabolic and physiologic responses observed in rodents and monkeys. These include reduced body weight, along with reductions in subcutaneous fat, visceral fat and lean muscle mass, reduced insulin and improved lipid profiles, reduced energy expenditure and core body temperature [40-50]. Assuming this type of dietary restraint is sustainable beyond the short term (six-month to two-year) duration of the study one would expect these physiological changes to predict a reduction in age-related disease. Researchers have also studied volunteers who have adopted a self-restricted lifestyle and maintained it for longer durations than the current CALERIE study [51–56]. In agreement with the research results from animal studies, voluntary CR in humans results in significant improvements in cardiovascular profiles, glucose control, body composition and circulating hormones [51–

56]. The combined results from these randomized control studies and the self-restriction groups demonstrate that CR has beneficial short-term physiological effects in humans, particularly contributing to a reduction in cardiovascular and metabolic-related diseases risk factors, conditions which account for a significant proportion of the healthcare related costs and morbidity/mortality in the US [57;58]. Whether long-term CR would significantly increase longevity in humans will likely remain a matter of debate. The advancement of alternative restriction paradigms may ultimately aid in understanding this potential. Two types of DR, alternate day/intermittent fasting, sometimes called every other day feeding, and single nutrient restriction (e.g. protein or methionine restriction), are increasingly reported to produce positive health and longevity benefits similar to sustained, daily CR [59–68]. Although current data are intriguing and suggestive of potential implications for human health, these interventions also lack a clear, defined mechanism of action, much like CR. Future studies in a variety of organisms with varied dietary compositions will be necessary to further validate the significance of these findings.

Is there a short-cut to CR? Progress in mimetics research

As it is impractical and of questionable desirability to maintain long-term CR, starting in early life in humans and sufficient to produce beneficial effects on health and longevity commonly observed in the laboratory models, other alternatives have been pursued. It is proposed that identifying the genetic and physiological mediators of CR could aid in the discovery of compounds/treatments that would act on those pathways, thereby mimicking the positive aspects of CR without imposed food restriction [24;69–73]. An ideal calorie restriction mimetic (CRM) is proposed to: i) produce metabolic, hormonal and physiological effects similar to CR, ii) not induce a significant reduction of long-term food intake, iii) activate stress response pathways similar to CR iv) while providing beneficial effects on mortality and age-related disease [70]. This area of research has progressed rapidly over the past decade as large-scale genomic, proteomic and metabolomic studies are performed in model organisms, attempting to unravel the complex interactions of genetics and nutrition that regulate aging and disease [74–85]. The National Institute on Aging has established the Interventions Testing Program (http://www.nia.nih.gov/ResearchInformation/ScientificResources/InterventionsTestingProgram.htm) as a multi-institutional program to collaborate with partner researchers and test substances predicted to “extend lifespan and delay disease and dysfunction” [86–91]. Thus far approximately a dozen agents have been investigated in the program, yielding both promising and mixed results (see Table II). The structure of the program utilizes three separate test sites (the University of Michigan, the Jackson Laboratories and the University of Texas Health Sciences Center at San Antonio), with each candidate compound being tested for longevity effects in a total of 108 female and 132 male mice (n=36/F, 44/M per site) and untreated control groups with twice as many mice (n=72/F, 96/M per site) [87;88]. This provides a sufficient sample size to detect a 10% change in mean lifespan with 80% power even if data from one site is for some unforeseen reason unusable [87;88;90]. Results from one of the most recent test compounds, rapamycin, are discussed below.

Resveratrol

Of the CRMs thus far investigated, few have received more attention than resveratrol. Resveratrol is a plant-derived polyphenol, most well known for its presence in the skins of red grapes. Studies in yeast, worms and flies over the past decade have suggested that CR works by activating members of the Sirtuin family of protein de-acetylases to mediate the lifespan benefits [92–96]. Resveratrol is reported to activate Sir2 [97], thus mimicking the benefits of CR in the absence of actual nutrient alteration [97]. Additionally, treatment with resveratrol is reported to mimic CR by increasing lifespan in yeast, worms, flies and fish, potentially through the activation of sirtuins [97–100]. However, the fundamental role of the

sirtuins in mediating the benefits of CR in yeast has been challenged by demonstrations that CR can extend lifespan in the absence of Sir2 or other sirtuins [101–104], while the *in vivo* activation of Sir2 by CR or resveratrol to extend lifespan has been challenged in multiple organisms [105–113]. Recent reports have expanded previous work which showed the *in vitro* activation of SIRT1 by resveratrol is substrate specific, challenging the basic mechanism of sirtuin activating compounds currently being tested [105;114–116].

Despite these disparate data, it appears resveratrol treatment produces a transcriptional response similar to CR [117], and in the presence of a high-fat diet, both health and longevity benefits have been reported [118]. However, when resveratrol was added to a normal diet, no significant lifespan benefits were observed in mice [119], suggesting it is not a true CRM. Based on the current data, resveratrol supplementation produces a variety of physiological benefits [120], but whether these are mediated by the sirtuins and are a *bona fide* mimic of CR is questionable and will require further data and clarification [105;114–116].

Rapamycin

Rapamycin (RAP), another proposed CRM, is an antibiotic and inhibitor of TOR (Target of Rapamycin) signaling in cells, with known immunosuppressive and anti-proliferative effects [121]. TOR has been identified as a mediator of nutrient signaling in cells, and is proposed to play a role in aging and the CR response [121–131]. A recent ITP study reported a significant mean lifespan extension in both male (9%, $P<0.0001$ [log-rank test]) and female (13%, $P<0.0001$ [log-rank test]) mice fed a standard diet and administered RAP beginning at approximately 20 months of age [90]. This is the first compound to provide such robust lifespan benefit in the ITP. Interestingly, CR is usually initiated prior to 6 months of age, and although CR can extend lifespan even when started at older ages [1;132] the effect at older ages is less pronounced and less reliable [3]. Notwithstanding the increase in lifespan, no significant differences were observed in the distribution of lesions found at necroscopy with RAP treatment, suggesting the longevity benefits of RAP treatment may be mediated by pathways partially independent of the normal CR response [90]. However, no measures of glucose, insulin or body temperature were reported to permit a comparison of RAP treatment with the expected results of CR, although body weight was not reduced with RAP treatment [90]. The authors recognized and reported differences in the composition of the pre-study diets, which although all were based on the same standard (NIH-31), varied in the specific formulations [90]. Nevertheless, one of the test sites (The Jackson Laboratory) utilized the control diet for the duration of the study and observed a significant increase in lifespan in both male ($P=0.02$ [log-rank test]) and female ($P<0.0001$ [log-rank test]) mice [90]. An additional cohort of mice with RAP treatment initiated at 9 months of age will likely further validate the potential benefit of RAP on lifespan [90]. A fundamental question that arises from these results is whether CR, when combined with RAP treatment, would provide additional health and lifespan benefits, particularly if RAP is acting on the pathways mediating CR's longevity effect? Likewise, these results should be tempered with the reality that RAP is used as an immunosuppressant, of limited consequence in rodent longevity studies since mice are maintained in specific pathogen free facilities. However, its utility for administration to healthy humans, which rely on a robust immune system in daily life, is currently unclear.

Other Potential CRM

Metformin—A hallmark of the CR response is reduced circulating glucose and insulin, while the role of insulin/IGF-1 in aging has received much support from model organism studies [133–137]. Therefore, it was proposed that drugs that could reduce insulin and glucose would be potential CRM candidates [24;70;133]. The biguanide metformin is used

in the treatment of diabetes where it functions to suppress gluconeogenesis and increase insulin sensitivity [138], suggesting it could mimic CR. Metformin is also reported to partially mimic the CR transcriptional response in mice [80] and increase median lifespan in *C. elegans* [139]. In addition, a number of studies have shown that metformin and related biguanides, phenformin and buformin, delay the incidence and development of cancers and other disease conditions [134;140–149]. However, a test of metformin as a CRM with a normal diet and in a non-disease rodent model has not been reported. To address this deficiency, a longevity study of healthy male Fischer-344 rats fed a standard diet with metformin supplementation (300 mg/kg/day) has been performed. Metformin did not significantly increase lifespan compared to control rats (unpublished data), although only one dose of metformin was tested and the CR group did not extend maximum lifespan in the study. Therefore, we await data showing a significant lifespan benefit in the absence of a disease state with metformin supplementation before a final verdict regarding its status as a true CRM.

2-Deoxyglucose—2-Deoxyglucose (2DG) is a non-metabolizable glucose analogue, which is taken up by cells where it accumulates while inhibiting glycolysis [69;133]. Thus, 2DG is proposed to reduce the metabolic flux of glucose, resulting in a reduced energetic flow in cellular metabolism resembling CR [69;133]. However, one difficulty with 2DG is the dose dependent inhibition of basic cellular function, resulting in toxicity with increasing concentration [70;133]. Preliminary experiments with 2DG supplementation resulted in lowered plasma insulin and body temperature, similar to CR [133]. When administered long-term by diet supplementation (either 0.2% or 0.4% 2DG) in male Fischer 344 rats, rather than increasing lifespan, a dose-dependent reduction in lifespan was observed [150], suggesting there is a fine line between pharmaceutically mimicking the effects of CR with 2DG without causing toxicity and death [72].

Longevity and Quality of Life, do we desire one without the other?

Should we be concerned about the potential for increased human lifespan? Clearly life expectancy has risen over the last century and appears to be continuing to do so, although disagreement exists regarding the potential of future increases [151–156]. However, some reports warn of a potential plateau or impending reversal of these lifespan gains in developed countries as a result of multiple factors, perhaps most notably the current obesity epidemic [157]. What is of most concern is the potential that lifespan will increase, while the onset of age-related disease and co-morbidity will remain the same, resulting in the unpleasant outcome of reduced quality of life for a greater duration in old age. Although much of aging research has focused on lifespan, the number of days of life until death, an alternate measure of aging termed the healthspan, or the length of time prior to the onset of age-related disease, has been considered and may be of particular importance to humans as life expectancy continues to increase [158–161]. The results from the Wisconsin Rhesus monkey CR study [12], as well as other nonhuman primate studies, suggest even if longevity benefits are realized with CR, they may be secondary to the health gains achieved. Thus, the academic or esoteric question of whether lifespan can be truly extended by CR in humans may not be as important as the potential prolongation of healthspan. If these types of health-promoting and disease-reducing results can be achieved in humans, as short-term CR studies suggest, the answer may be that quality of life can be extended, potentially into advanced age. Likewise, the search for CRMs that extend lifespan, without altering the underlying disease pattern, would be of little utility. Therefore, careful examination of multiple outcomes beyond lifespan should be considered in any CRM intervention study to assess the effect on disease. Further study to identify the central pathways which mediate the beneficial responses of CR should be of high priority as these may serve as useful targets for interventions to improve health and possibly lifespan.

Concluding Remarks

The recent reports of Colman et al. (2009) and Harrison et al. (2009) illustrate the potential of translating fundamental discoveries across organisms in the effort to retard aging and disease. Due to the nature of a longevity study in primates, the final answer regarding the effect of CR on total and maximal lifespan is still probably a decade away. Whether these types of interventions will reduce disease incidence/severity and increase lifespan in humans is still unknown. Nevertheless, these results are pointing in a positive direction and suggest that finding a means to implement or mimic the CR response in humans could significantly affect the health and well-being of our species, regardless of the eventual lifespan result.

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Table I

Abbreviations

AL	*Ad libitum*
CALERIE	Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy
CR	Calorie Restriction
CRM	Calorie Restriction Mimetic
2DG	2-Deoxyglucose
ITP	Interventions Testing Program
NIA	National Institute on Aging
RAP	Rapamycin
TOR	Target of Rapamycin

Table II

Interventions Testing Program Compounds

Compound	Concentration	Age of Treatment Initiation	Lifespan Effect Sex – Mean Effect* (p-value)	Description	Reference
Aspirin	20 ppm	4 months	M – 8% (p=0.001) F – *ns*	Non-steroidal anti-inflammatory, anti-thrombotic, anti-oxidant	[1;2]†
Nitroflurbiprofen (NFP)	200 ppm	4 months	M – *ns* F – *ns*	Nitric oxide releasing non-steroidal anti-inflammatory drug	[1;2]†
Nordihydroguaiaretic acid (NDGA)	2,500 ppm	9 months	M – 12% (p=0.006) F – *ns*	Anti-oxidant, anti-inflammatory, polyphenol	[1;2]†
4-OH-α-phenyl-tert-butylnitrone (4-OH-PBN)	315 ppm	4 months	M – *ns* F – *ns*	Nitrone-based free radical trap	[1;2]†
Caffeic acid phenethyl ester (CAPE)	30 ppm	4 months	M – *ns* F – *ns*	Anti-oxidant, anti-inflammatory, anti-tumorigenic	[3]†
Caffeic acid phenethyl ester (CAPE)	300 ppm	4 months	M – *ns* F – *ns*	Anti-oxidant, anti-inflammatory, anti-tumorigenic	[3]†
Enalapril Maleate	120 ppm	4 months	M – *ns* F – *ns*	Anti-hypertensive agent; Angiotensin converting enzyme inhibitor	[3]†
Rapamycin	14 ppm	20 months	M – 9% (p<0.0001) F – 13% (p<0.0001)	Anti-fungal, anti-cancer, immunosuppressant	[3]†
Rapamycin	14 ppm	9 months	*In testing*	Anti-fungal, anti-cancer, immunosuppressant	[3]†
Simvastatin	12 ppm	10 months	*In testing*	Hypolipidemic drug	†
Simvastatin	120 ppm	10 months	*In testing*	Hypolipidemic drug	†
Resveratrol	300 ppm	12 months	*In testing*	Phytoalexin, anti-fungal, anti-microbial, anti-cancer, anti-inflammatory, polyphenol	†
Resveratrol	1,200 ppm	12 months	*In testing*	Phytoalexin, anti-fungal, anti-microbial, anti-cancer, anti-inflammatory, polyphenol	†
Resveratrol	300 ppm	4 months	*In testing*	Phytoalexin, anti-fungal, anti-microbial, anti-cancer, anti-inflammatory, polyphenol	†
Oxaloacetic acid	2,200 ppm	4 months	*In testing*	Citric acid cycle metabolite	†
Green Tea Extract	2,000 ppm	4 months	*In testing*	Anti-oxidant, anti-inflammatory, polyphenol(s),	†
Curcumin	2,000 ppm	4 months	*In testing*	Anti-tumor, anti-oxidant, anti-inflammatory, polyphenol	†
Medium Chain Triglyceride Oil	60,000 ppm	4 months	*In testing*	Fat supplement	†
17α-Estradiol	4.8 ppm	10 months	*In testing*	Neuroprotective, mitochondrial protective, muscle relaxant	†
Methylene Blue	28 ppm	4 months	*In testing*	Chemical dye, diverse biological activities	†
Acarbose	1,000 ppm	4 months	*In testing*	Glycoside hydrolase inhibitor, anti-diabetic	†

Compound	Concentration	Age of Treatment Initiation	Lifespan Effect Sex – Mean Effect* (p-value)	Description	Reference
Rapamycin LoPhase II	4.7 ppm	9 months	*In testing*	Anti-fungal, anti-cancer, immunosuppressant	†
Rapamycin MidPhase II	14 ppm	9 months	*In testing*	Anti-fungal, anti-cancer, immunosuppressant	†
Rapamycin HiPhase II	42 ppm	9 months	*In testing*	Anti-fungal, anti-cancer, immunosuppressant	†

M=Male, F=Female; *ns* – not significant

*Log-rank test for lifespan effects; significance set at p<0.05[1–3]

†http://www.nia.nih.gov/ResearchInformation/ScientificResources/CompoundsInTesting. htm