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Review Article

Dietary Interventions to Extend Life Span and Health Span Based on Calorie Restriction

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The societal impact of obesity, diabetes, and other metabolic disorders continues to rise despite increasing evidence of their negative long-term consequences on health span, longevity, and aging. Unfortunately, dietary management and exercise frequently fail as remedies, underscoring the need for the development of alternative interventions to successfully treat metabolic disorders and enhance life span and health span. Using calorie restriction (CR)—which is well known to improve both health and longevity in controlled studies—as their benchmark, gerontologists are coming closer to identifying dietary and pharmacological therapies that may be applicable to aging humans. This review covers some of the more promising interventions targeted to affect pathways implicated in the aging process as well as variations on classical CR that may be better suited to human adaptation.

Key Words: Aging intervention—Health span—Life span—Metformin—Resveratrol.

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As the quincentennial of the 1513 launch of Ponce de Leon's famously futile quest for the fountain of youth draws near, efforts of gerontologists over the past several decades have identified few therapies that consistently extend the life span of multiple species. Calorie restriction (CR), the reduction in macronutrient intake while maintaining sufficient micronutrient intake, is one notable exception. Early studies by McCay and colleagues (1–3) established the effectiveness of CR for extending the life span of rats in the 1930s, and subsequent studies have demonstrated that sustained reductions in calorie intake can increase maximum life span in a wide range of species (4).

To date, CR remains the most robust dietary intervention in aging research, and because CR is so successful at promoting health and longevity in laboratory animals, there is increasing interest in the therapeutic potential for CR to extend life span in humans (5). Furthermore, as emerging cellular mechanisms responsible for aging continue to demonstrate considerable overlap across species, there is increasing evidence that intervention strategies can be effectively evaluated in short-lived animals as a screen for potential human therapies (6). Although there is debate as to whether CR will function in humans as effectively as it does in shorter lived research models (7,8), nonhuman primate data suggest that CR can improve at least the quality of life in our close relatives (9). Regardless, the prospect of CR in humans is already a reality with societies, books, and Internet sites devoted to CR in humans (see http://www.calorierestriction.org/). Despite willful adherence by CR devotees, gerontologists believe that most humans will prefer not to restrict their diet in the presence of an abundant food supply if alternatives, or calorie restriction mimetics (CRM), can be identified.

The ideal CRM would be an agent consumed in food or water that would delay death and age-associated diseases without requiring a change in calorie intake (5). Candidate CRM are already under investigation in rodent models (5,10), with the ultimate goal being to translate the research findings to a therapy applicable to humans. A human aging intervention would be particularly timely as the baby boomer generation approaches retirement age. Fortunately, CR has been shown to increase life span even when applied late in life (11), so a true CRM would also function for older humans and offer tangible benefits to consumers of middle age and beyond.

This review will explore the most current data on dietary aging interventions actively being tested for their ability to extend life span and health span. Because the specific mechanisms underlying aging are not known, and neither are the mechanisms by which CR forestalls aging known, there are presently many compounds being tested that have varying physiological targets. It should be noted that although the following treatments show promise in certain animal models, evidence for these interventions from randomized trials with humans is extremely weak to nonexistent. The compounds are discussed within their general purported mechanism of

action against the aging process. Protein restriction (PR) and intermittent fasting (IF) feeding, two dietary restriction techniques that are similar to CR but do not require a reduction in overall caloric intake every day, are also reviewed.

Antioxidants

One of the original theories of aging—the free-radical theory of aging—proposes that reactive oxygen species (ROS), the highly reactive by-products of daily cellular metabolism that can damage other molecules and cell structures, might drive the aging process (12). Toward this theory, much research has focused on the potential for antioxidant compounds to forestall aging and age-related disease.

Although the ROS theory of aging continues to appeal to both the researchers and the general public, clinical trials have failed to demonstrate that food-based antioxidants prolong life or prevent age-related diseases. In fact, a meta-analysis of randomized trials involving more than 230,000 participants consuming beta-carotene, vitamin A, vitamin C (ascorbic acid), vitamin E, or selenium found no benefit to longevity with the supplements (13). Furthermore, the researchers concluded that supplementing one's diet with beta-carotene, vitamin A, and vitamin E may increase mortality. Focusing on age-related disease, another meta-analysis examined the use of dietary antioxidants for the prevention of age-related macular degeneration (14). The study considered nine prospective cohort studies including nearly 150,000 people and concluded that vitamin A, vitamin C, vitamin E, zinc, lutein, zeaxanthin, alpha-carotene, beta-carotene, beta-cryptoxanthin, and lycopene have little or no effect in the primary prevention of the disorder. Although avoiding deficiencies of these vitamins is essential for normal physiological functioning, increased intake shows no increased health benefits and in some cases may have toxic effects. Taken together, the current data do not support long-term antioxidant supplementation in humans.

Glucose and insulin homeostasis

Among the plethora of physiological changes associated with aging are a decline in glucose tolerance and an increase in insulin resistance. Hyperinsulinemia, hyperglycemia, and insulin resistance are thought to contribute to several chronic disorders associated with aging, including diabetes, hypertension, cancer, and cardiovascular disease. CR has been shown to beneficially affect glucose and insulin levels, preventing and even reversing conditions associated with hyperglycemia and insulin resistance. Given these observations, there is hope that targeted CRM may be identified or developed to favorably affect health span and life span through their actions on glucose and insulin homeostasis.

*2-Deoxy-*D*-glucose.*—The glucose analog 2-deoxy-D-glucose (2DG), a compound that inhibits glycolysis, has been investigated as a potential CRM because its effects on energy metabolism may recapitulate some of the metabolic effects of CR (5). In fact, a number of studies have demonstrated that 2DG treatment in rodents produces a number of effects that parallel those of CR, such as reductions in body temperature, heart rate, and circulating glucose and insulin (5,15–17) and increases in circulating glucocorticoids and heat-shock proteins (16–18). Beyond these effects, 2DG has also been shown to confer functional benefits associated with CR, including inhibition of tumor growth (19,20) and increased stress resistance to neurotoxins and cold shock (17,21–23).

Regarding life-span extension, one study using *Caenorhabditis elegans* found that restriction of glucose metabolism by 2DG led to extensions in both mean and maximum life span (24). Despite these promising preliminary findings, the case of 2DG emphasizes the need for progressive screening of interventions through several species because our laboratory has recently reported that 2DG feeding in rats produces negative cardiotoxic effects and increased mortality associated with a deregulation of protein degradation and clearance (25). Although 2DG may still have therapeutic value over the short term in targeted applications such as chemotherapy and brain imaging, its prospects as an aging intervention are seriously diminished in light of its chronic effects in rats.

Biguanides.—Also of interest as aging interventions that improve glucose homeostasis are derivatives of biguanide compounds, first isolated from the French lilac, such as buformin, metformin, and phenformin. Although all three were effective treatments for diabetes mellitus and showed promising results in rodents in tumor inhibition (26), buformin and phenformin have been largely withdrawn from clinical practice due to association with lactic acidosis. Metformin, however, is still popularly prescribed as a treatment for type 2 diabetes. Metformin decreases hepatic gluconeogenesis (27,28) and increases insulin sensitivity (29). It is a potent activator of adenosine monophosphate–activated protein kinase (30) and thereby inhibits the mammalian target of rapamycin (mTOR) (31), a protein kinase that is involved in the control of cellular proliferation and is implicated in tumor growth (32,33). In mice, metformin extended mean and maximum life span in different female strains predisposed to high incidence of mammary tumors (34,35).

Like CR, metformin facilitates the entry of glucose into cells by increasing insulin sensitivity (36,37), and microarray studies have further revealed that metformin is capable of inducing gene expression patterns that closely resemble those of CR (38,39). In humans, metformin supplementation has been shown to reduce adiposity (40) and mortality rates in diabetic individuals, most effectively in obese and insulin-resistant individuals (41–43). Although the data so far are promising, further study is needed to show whether metformin is able to extend life span in healthy rodents, primates, and humans.

Advanced glycation end products

In the 1970s, researchers discovered that in diabetic individuals, excess glucose could combine with proteins (as happens to meat and bread products during cooking) yielding a sticky brown substance (44). These compounds were found to attract other proteins and congregate in a web-like network that could stiffen joints, block arteries, and cloud clear tissues like the lens of the eye, leading to cataracts (45). Given the considerable overlap between the complications of diabetes and general ailments associated with aging, it was unsurprising that glycosylated proteins were found to accumulate in normal aging tissues (46). Since then, considerable interest has been shown in the potential to identify or develop compounds that could inhibit or reverse the accumulation of these advanced glycation end products (AGEs) as a treatment for complications associated with both diabetes and aging.

The effects of AGE inhibitors on life span remain to be demonstrated; however, there have been promising in vitro reports of reduced senescence and increased replicative life span in cell cultures (47,48). Furthermore, one study found that reducing dietary exposure to preformed AGEs (achieved by reducing the exposure of the diet to heat during processing) extended both mean and maximum life span of mice (49). Even more intriguingly, a study of CR in rats reported reduced AGE accumulation in the animals with restricted food intake (50), and more recently, a study looking at the relationship between CR, AGE accumulation, and life span found that feeding mice a diet high in AGEs blocked CR's ability to extend mean or maximum life span (51).

Aminoguanidine.—The most well-studied AGE inhibitor, aminoguanidine (also known as pimagedine), has additional functions as a general antioxidant and inhibitor of the enzymes diamine oxidase and nitric oxide synthase (52–54). In rats, supplementation with aminoguanidine in the water supply prevented arterial stiffening and cardiac hypertrophy that correlated with a decrease in AGE-induced cross-linking of the extracellular matrix (55). Aminoguanidine treatment in mice was also able to block negative AGE-associated effects on the immune system (56). These positive effects may not translate to enhanced longevity, however, as one study found life span was unaffected by aminoguanidine supplementation in male mice (39).

Another issue with aminoguanidine regarding side effects was raised in a clinical trial investigating the effectiveness of aminoguanidine in the treatment of diabetic nephropathy that involved more than 450 patients (57). Although the researchers did find that compared with placebo-treated controls those taking aminoguanidine showed reduced progression of retinopathy and improved serum lipid profiles, the high dose was associated with adverse effects: ~9% of participants reported negative gastrointestinal effects and ~1% of patients developed autoimmune complications.

Pyridoxamine.—Another AGE inhibitor, pyridoxamine, is also able to bind intermediates of lipid peroxidation and thus prevent advanced lipoxidation reactions (58). Pyridoxamine is a member of the vitamin B_6 family, a water-soluble nutrient found in reduced levels in aged individuals (59,60). Although pyridoxamine has been shown to reduce cross-linking associated with cataract formation in mice (61), further studies are required to assess the extent to which pyridoxamine can protect against the harmful effects of glycation and free radicals in aging.

mTOR Signaling Pathway

Given the starkly opposing effects of excessive calorie intake versus restricted calorie intake on the aging process and life span, there has been considerable interest in understanding the role of molecular energy–sensing pathways in aging and how they may be manipulated to alter health span and life span. One key sensor of nutrient availability in higher organisms is mTOR. Inhibition of related pathways in worms, flies, and yeast has been shown to extend life span (62–64), and in mammals, mTOR has become increasingly understood as a central regulator of energy homeostasis and cellular metabolism (65). Given that CR has been shown to inhibit target of rapamycin signaling in multiple species including mice (62,66), mTOR has become a candidate mediator of at least some of CR's beneficial effects and agents that inhibit mTOR signaling are candidate CRM.

Rapamycin.—Rapamycin, also called sirolimus, selectively and effectively inhibits mTOR and also possesses an array of notable clinical effects, including antibiotic, antitumor, and immunosuppressant actions. Discovered in the 1960s in soil bacteria on Easter Island, dietary supplementation with rapamycin was recently found to extend life span in aged mice (67). Rapamycin is also being increasingly investigated for its potential to benefit age-related diseases like Alzheimer's, Huntington's, and Parkinson's diseases. These diseases, characterized by aggregate formation, may gain from mTOR's effects on protein turnover (68). A pharmacological derivative of rapamycin has also shown promising results in clinical cancer trials studying advanced renal cell carcinomas, glioblastomas, breast cancers, endometrial cancers, non-Hodgkin lymphomas, and multiple myelomas (69). Whether rapamycin or its derivatives will be safe and effective as human aging interventions is an unanswered question, and it may be that other effectors of the mTOR pathway yet to be identified will be more suited to long-term use in humans.

Sirtuin activators

It has been suggested that CR functions to extend life span at least in part through increasing the activity of sirtuins, a conserved family of proteins that includes human Sirt1 (70,71). The involvement of Sirt1 in life span extension by CR may relate to its responsiveness to nicotinamide

levels and the NAD^+:NADH ratio, both indicators of cellular energy status (72,73). Activation of Sirt1, the mammalian homolog of an NAD^+-dependent deacetylase known to modulate life span in lower organisms (74,75), is thought to hold promise as a strategy for delaying aging in mammals (76). Screening compounds for their ability to activate sirtuins first led to the discovery of resveratrol as a potential CRM (74). Although emerging evidence suggests that resveratrol and other compounds may not directly activate Sirt1, many beneficial health effects have been attributed to Sirt1, and its activation, whether direct or indirect, may be sufficient to promote health span and life span (77).

Resveratrol.—Resveratrol, a pluripotent polyphenol, is one of the more intuitively appealing CRM due to its relative abundance in red wine. Indeed, the so-called French Paradox describes the phenomenon that, despite a culture of high-fat diet and cigarette smoking, the French have been reported to enjoy relatively low mortality rates related to coronary heart disease, possibly due to high consumption of red wine (78). Resveratrol has been shown to enhance longevity in a number of short-lived organisms, including yeast, worms, flies, and the vertebrate fish *Nothobranchius furzeri* (74,79,80). Data from mice have also shown that resveratrol is able to mimic key aspects of CR, including gene transcription profiles, glucose sensitivity, and physical endurance (81–85). Regarding effects on life span, so far, resveratrol has not been shown to extend maximum life span of mice on a standard diet but only mice on a high-fat high-calorie diet (82).

However, promising research indicates that resveratrol, especially in combination with other compounds, could serve as a treatment or preventative measure against a wide variety of age-related ailments. In mice, resveratrol delays classic signs of age-related deterioration (82) and, in combination with quercetin and catechin, inhibits mammary tumor growth and metastasis to the liver and bone (86). Consumption of a similar cocktail of grape polyphenols improves endothelial function over the short term in patients with coronary heart disease (87). In one case study, resveratrol significantly improved visual and mental function in a male with age-associated eye degeneration (88). Further evidence demonstrates resveratrol's neurological benefits. In rats, resveratrol and 4-amino-1,8-naphthalimide (4-ANI, an inhibitor of an enzyme known to be hyperactive in diabetic neuropathy) together have been shown to partially reverse the effects of diabetic neuropathy, a major cause of death in diabetic patients (89). Resveratrol reduces infection-related neuroinflammation and attenuates working memory deficits in aged mice, suggesting that resveratrol may be useful for mitigating acute cognitive disorders in elderly individuals with an infection (90). Furthermore, although moderate red wine intake has been associated with reduced incidence of Alzheimer's disease and other forms of dementia (91–93), clear evidence regarding resveratrol's role is still lacking (94,95). Thus, although resveratrol may not be able to extend overall life span in healthy individuals, it will be important to assess the degree to which it might be able to increase quality of life among the overweight and the elderly individuals by mitigating some of the negative effects of an unhealthy lifestyle.

Synthetic sirtuin activators.—More recently, synthetic compounds structurally distinct from resveratrol have been described with potent Sirt1-activating power in vitro (96). One of the compounds, SRT1720, has been shown to mitigate various negative effects of obesity and high-fat diets in both rats and mice (96–98). SRT1720 has also been shown to induce a transcriptional profile in mice reminiscent of CR, eliciting parallel changes in genes associated with mitochondrial biogenesis, metabolic signaling, and inflammatory pathways (99). At this time, the long-term effects of these relatively novel compounds on health span and life span remain to be demonstrated.

Dietary restriction alternatives to cr

The current research paradigm of CR usually entails food intake restriction ranging from 20% to 40% on a daily basis. Because such extreme dieting over the long term is likely to be unrealistic in humans, there is interest in adapting the principles of CR to a regimen that is more palatable to humans and better suited to practical application.

Protein restriction.—Historically, the increase in life span seen with CR has been attributed to the overall reduction in calories and not to a reduction in an individual nutrient class (100). More recently, studies are emerging that suggest variations in the proportions or quality of the individual dietary components (especially the macronutrients) can also modulate health and consequently longevity independently of overall caloric intake. In particular, PR, where a percentage of calories derived from protein is replaced by fat or carbohydrate, has been investigated in controlled studies with varied results (101).

While several rodent studies have shown increases in life span with colonies maintained on low-protein diets (102–106), another found that PR was able to mimic CR in slowing growth and improving renal function, but the effects did not translate to increased life span (107). Furthermore, a study of spontaneous tumor formation in rats concluded that a low-protein regimen actually increased mortality (108). Another issue with some PR studies is that they also report a natural decrease in food consumption by animals on PR, obscuring the distinction between PR and CR and preventing the clear delineation of each regimen's effects.

Beyond total PR, there is also evidence that restriction of specific amino acids can affect health and longevity. For example, rats fed tryptophan-deficient diets exhibit delayed sexual maturation, delayed tumor onset, improved hair growth and coat condition, and increased maximum life

span (109–111). It should be noted, however, that one rat study found increased mortality during the juvenile period and consequently an overall decrease in mean life span (112). This negative effect on early mortality was not observed in mice on a tryptophan-depleted diet, and the mice showed an increase in mean and maximum life span and no differences in food intake (113). Thus, tryptophan's effects on juvenile survival need further study. Beyond this, the side effects of tryptophan depletion in humans also need to be assessed as this essential amino acid is the precursor for the neurotransmitter serotonin. In humans, depleted serotonin levels can lead to psychological disturbances and increased risk of suicide (114), which could negate any life extension effect of tryptophan restriction.

Methionine restriction has also been reported to benefit health and longevity in animal studies. Decreased mitochondrial ROS, serum glucose, insulin, and insulin-like growth factor-1 levels and increased mean and maximum life span have been reported in rats and mice maintained on diets restricted in methionine (115–120). However, as with CR, rodents consuming low-methionine diets are smaller and eat less (10%–24% less, depending on age) than controls (117,121,122). As this continues to be a recurring issue with protein- and amino acid–restrictive diets, a few studies have attempted to show that the benefits of methionine restriction are independent of any concomitant CR. One study showed that methionine restriction results in decreased visceral fat deposition and preservation of insulin sensitivity, and these effects were not seen in pair-fed controls (117). Another study achieved consistent caloric intake among control and experimental animals through use of a methionine-depleted energy-dense diet and found that methionine restriction reduced body size regardless of equivalent caloric intake (123). These results suggest that growth impairment can be related to methionine intake and not solely calorie intake, but it still remains to be shown that methionine restriction independently of any CR effect results in life-span extension in rodents.

The impact of PR, tryptophan restriction, or methionine restriction on human health and longevity remains to be evaluated. Beyond the general hurdle of the Western predilection for high-protein intake, the feasibility of designing long-term human diets that are restricted in specific amino acids may be low at least in the near future. More research into the effects of protein and amino acid restriction in humans is warranted, given that vegetarian populations enjoy reduced risk for chronic diseases of aging (124), and this may be attributable at least in part to reduced protein intake.

Intermittent fasting.—Another approach to adapting CR to humans may involve adjusting the term of CR or IF. In mice, IF administered as 30%–50% CR over periods of 2–3 weeks followed by ad libitum feeding for an equal amount of time has been shown to be protective against tumor formation in models for both prostate cancer and breast cancer (125,126). In humans, IF has been shown to be both attainable and beneficial to health (127,128). For example, overweight participants participating in an 8-week study using IF (participants alternated daily between ad libitum intake and 80% CR) lost weight and increased in measures of pulmonary function, perceived energy, and mood (127). Importantly, several recent short-term interventions have examined IF in nonobese humans (129–131) and have found significant health benefits that parallel CR, including increased insulin sensitivity (127), upregulation of Sirt1 (128), and decreased fat mass (131). Although the potential for IF to confer short-term health benefits to humans seems significant, IF's effects on the aging process are not known. Moreover, the long-term implications of IF warrant careful consideration as some human observational studies have drawn an association between so-called yo-yo dieting and increased morbidity and mortality (132).

Conclusions

Currently, there are no known interventions proven to substantially slow the aging process in humans. Indeed, it has been argued that such drugs can never be developed because aging is caused by a random accumulation of damage, some of which is inevitable and irreversible (133). Even further, although much of the research over the past several decades has focused on CR, currently, there are no conclusive data showing long-term health span or life-span benefits from CR in humans. Nevertheless, ongoing research continues to draw a more complete picture of CR at the molecular level, which may ultimately allow for the development of therapeutics that might be able to confer at least some of the health benefits of this dietary regimen. Such developments would be particularly appealing to aging populations in developed countries as continuous warnings from health care institutions and governments are ignored in favor of increased calorie consumption and decreased physical activity. Furthermore, compounds that slow the aging process by forestalling age-related disease would not only lengthen life but improve the quality of life as well.

As researchers continue to elucidate the mechanisms of aging and their underlying molecular pathways, the range of targets for aging interventions should continue to increase. Indeed, ongoing research in the field continues to highlight promising compounds that warrant further study (10,134,135). A significant portion of this wealth of data is emerging from a program initiated in 2003 by the National Institute on Aging (NIA) to rigorously test pharmacological and dietary agents that may extend the longevity of mice (10,136) (http://www.nia.nih.gov/ResearchInformation/ScientificResources/CompoundsInTesting.htm). The studies coming out of the collaborative efforts spearheaded by the NIA along with results from independent laboratories should continue to bring credit to the field of genuine aging research.

That said, it is important to emphasize that all the compounds currently under study have not been definitively shown to effectively reduce aging or age-related disease in humans. Despite this fact, a vast array of products are currently being sold to consumers with claims that it is now possible to slow, stop, or reverse human aging. Although the business of antiaging medicine is a lucrative multimillion-dollar industry, the products being sold have no demonstrated efficacy. It becomes the responsibility of researchers in the field of aging to educate the public on the distinction between the pseudoscientific antiaging industry and the genuine science of investigating aging interventions. Fortunately, the aging field has progressed rapidly in recent years and continues to hold promise for improving the quality and quantity of human life in the foreseeable future.

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