REVIEW SUMMARY

AGING

Antiaging diets: Separating fact from fiction

Mitchell B. Lee, Cristal M. Hill, Alessandro Bitto, Matt Kaeberlein*

BACKGROUND: Reduced caloric intake without malnutrition is the oldest known life span–extending intervention. Laboratory studies throughout the 20th century established and confirmed the benefits of caloric restriction (CR) in multiple model systems. CR not only increased life span across evolutionarily distant organisms but also reduced age-associated disease burden and functional decline in these studies. Epidemiological data from human populations is also generally consistent with the idea that lower caloric intake is associated with increased life expectancy. In recent years, numerous diet modalities that are purported to be "antiaging" have sprung from these observations. These diets restrict particular macronutrients (carbohydrates or protein) or feeding intervals and can be divided into those that impose reduced caloric intake versus those that are isocaloric to control diets.

ADVANCES: We evaluated several of the most popular antiaging diets, including CR, intermittent fasting, fasting-mimicking diets, ketogenic diets, time-restricted feeding, protein restriction, and essential amino acid restriction. By characterizing these nutritional interventions in comparison with classical CR, we gained numerous insights. Many studies fail to control for reduced caloric intake in the diet group, making their effects impossible to decouple from CR. Although often presented as uniformly beneficial, the effects of CR on life span are highly dependent on genotype and, in some cases, cause reduced survival. Despite their limitations, these studies have greatly improved our understanding of the cellular response to low nutrient availability. A picture is beginning to emerge of a complex network composed of multiple signaling pathways that converge on key molecular hubs; foremost among these is the mechanistic target of rapamycin (mTOR). Because mTOR and other components of this network are well-studied drug targets, there continues to be considerable interest in pharmacologically targeting this network to increase longevity and health span. Human studies, both correlative and controlled, are consistent with health benefits conferred by a CR diet. However, it remains unresolved whether these benefits are a consequence of modulating the aging process itself or are simply the result of avoiding obesity. Several unresolved questions suggest caution when considering whether to recommend or implement any of these diets among the healthy general public. Among these is understanding how genetic and environmental variation modify diet response, especially in understudied populations and in the context of environmental challenges such as, for example, a global viral pandemic.

OUTLOOK: CR and other antiaging diets have yielded important insights into the complex and evolutionarily conserved signaling pathways that transduce information regarding environmental nutrient availability into a physiological response to promote healthy longevity. This understanding, in turn, has opened the door to a new generation of longevity-promoting interventions that mimic molecular responses to nutrient deprivation. Although CR and other diets hold promise, additional data from carefully controlled studies is needed before broadly recommending or implementing these diets, or other interventions, for otherwise healthy people. Human genetic and environmental variation combined with the challenge of modeling human aging in ultimately dissimilar mammalian model systems pose fundamental limitations to our current ability to predictably translate these findings to people. From a pragmatic perspective, even if these challenges can be overcome, widespread adoption of dietary interventions for healthy longevity seem unrealistic. We therefore suggest that alternative, nondietary strategies with the potential for public uptake should therefore be pursued. In particular, validated biomarkers of biological aging are required to match intervention to each person's distinct genetic and environmental context and thereby optimize individual healthy life span. Future research directed at clarifying the underlying mechanisms involved in eliciting the longevity-promoting response to CR, and how this differs among individuals, should one day help us realize a true precision geroscience approach. ■

IMAGE: KELLIE HOLOSKI/*SCIENCE*

At the buffet of antiaging diets, which is the best plate? Diets clockwise from top left: CR, time-restricted feeding, protein restriction, and ketogenic.

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Caloric restriction has been known for nearly a century to extend life span and delay age-associated pathology in laboratory animals. More recently, alternative "antiaging" diet modalities have been described that provide new mechanistic insights and potential clinical applications. These include intermittent fasting, fasting-mimicking diets, ketogenic diets, time-restricted feeding, protein restriction, and dietary restriction of specific amino acids. Despite mainstream popularization of some of these diets, many questions remain about their efficacy outside of a laboratory setting. Studies of these interventions support at least partially overlapping mechanisms of action and provide insights into what appear to be highly conserved mechanisms of biological aging.

Modern aging research can trace its roots back to studies from the early 1900s that examined the effects of reduced food intake on life span in rats (*1*, *2*). These pioneering experiments showed that reducing caloric intake in laboratory-reared animals delays development and results in a substantial increase in adult life span. Work by Weindruch, Masoro, Walford, and others in the 1970s, 1980s, and 1990s expanded and popularized this area of research in both rats and mice and established caloric restriction (CR) as the dominant paradigm for antiaging intervention (Box 1) for the rest of the 20th century (*3*). These foundational studies provided strong evidence that CR not only increases life span in rodents but also reduces disease burden and delays many functional declines of old age (*4*).

A common definition for CR in these early studies is "reduced caloric intake in the absence of malnutrition" (*5*). Typically, this was accomplished by limiting chow by a fixed amount while supplementing with vitamins and micronutrients. The precise method of food limitation varied (for example, intermittent versus continuous feeding), as did the timing of initiation (pre- or post-weaning) and degree of restriction. The data from these studies support the idea that at least in some common laboratory strains of both mice and rats, total caloric intake correlates inversely with life span up to about 50 to 60% restriction (so long as essential nutrition is maintained), and starting earlier in life gives larger effects on life span than starting later in life (*5*).

With popularization of invertebrate models in aging research near the end of the 20th century, it was natural for scientists to test whether CR could similarly affect aging in these organisms. Because culture conditions differ across species, multiple alternative methods of nutritional intervention were tested and found to increase life span. We will refer to these interventions collectively using the term "dietary restriction" (DR), which includes both sugar and amino acid limitation in budding yeast, reduced bacterial food availability in nematode worms, and lower levels of protein (yeast extract) or sugar in fruit flies (*6*). These foundational studies allowed for mechanistic analyses not previously feasible in rodents and identified a highly conserved nutrient-sensing, growth-promoting network that appears to regulate biological aging in many different organisms. Among the key proteins in this network are the mechanistic target of rapamycin (mTOR), adenosine 5′-monophosphate (AMP)–activated protein kinase (AMPK), insulin and insulin-like growth factor 1 (IGF-1)–like receptors, FOXO-family transcription factors, and nicotinamide adenine dinucleotide (NAD)–dependent sirtuin deacetylases (*7*, *8*).

As the molecular underpinnings of CR, and DR more generally, became established, attention shifted toward identifying small molecules that mirror the effects of CR on life span and health without requiring reduced food consumption. Such "CR mimetics" include the mTOR inhibitor rapamycin, the antidiabetes drug metformin, the glycolytic inhibitor 2-deoxyglucose, the intestinal α-glucosidase inhibitor acarbose, and sirtuin-activating compounds (*9*, *10*). Most putative CR mimetic compounds, with the possible exception of rapamycin, have thus far failed to match the magnitude of life span extension and health span benefits of CR. For example, metformin (a nonspecific AMPK activator) and resveratrol (a nonspecific sirtuin activator) primarily improve measures of metabolic health during aging in mice, including increased insulin sensitivity and reduced cancer incidence, without reproducibly extending life span (*11*, *12*). These discrepancies likely reflect a still incomplete understanding of the varied and diverse effects of CR, which have yet to be fully recapitulated pharmacologically.

The rise of antiaging diets

The goal of this critical Review is to summarize the current state of the field with respect to the most commonly studied antiaging dietary interventions, with a focus on potential shared mechanisms of action, important unanswered questions and areas for future inquiry, and addressing common misconceptions in the literature. We largely restrict our considerations to preclinical studies in rodents and, where applicable, relevant human data. For detailed reviews of CR in rodents, we refer the interested reader to these resources (*4*, *5*, *13*–*15*). Here, we only briefly discuss the evidence for antiaging effects of classic CR and instead focus on placing alternative dietary interventions into context with what is known about CR and potential impacts on human health and longevity. Among the interventions we consider are ketogenic diets (KDs), intermittent fasting (IF), fasting-mimicking diets (FMDs), time-restricted feeding

Box 1. Reclaiming the term "antiaging."

The phrase "antiaging" is greatly abused in popular culture, often for the purpose of marketing cosmetic procedures or unproven nutritional supplements purported to slow or reverse aging. This has the unfortunate consequence of creating confusion among the general public and diminishing the impact of legitimate scientific discovery. Here, we define "antiaging" as delaying or reversing biological aging by targeting the established molecular mechanisms of aging, which have been formalized as "hallmarks" or "pillars" of aging (*93*, *94*). Effective antiaging interventions in laboratory animals increase both median and maximum population life span and broadly delay the onset and progression of many age-related functional declines and diseases. The latter effect is often referred to as "extending health span," which is a qualitative term referring to the period of life free from chronic disease and disability (*95*). Recent studies show that at least some antiaging interventions, such as the drug rapamycin, can reverse functional declines across multiple tissues in aged animals (*96*). On the basis of this definition, there are as yet no clinically validated antiaging interventions in humans. However, there is some evidence consistent with antiaging effects for CR and related diets in humans as well as a small number of putative geroprotective compounds, including metformin and rapamycin (*97*).

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(TRF), protein restriction (PR), and diets restricted for specific amino acids, including methionine restriction, tryptophan restriction, and branched chain amino acid (BCAA) restriction.

One critical but often overlooked (and therefore quite confusing) consideration when evaluating different antiaging diets is the relative caloric intake of control and experimental cohorts. Antiaging diets can be considered in two broad groups: CR and isocaloric nutrient restriction (Table 1). Several of the most prominent antiaging diets such as IF, FMDs, and KDs generally fall under the CR umbrella because the experimental group typically consumes 20 to 40% fewer calories than does the control group. This makes evaluating the effects of dietary composition challenging to differentiate from the effects of reduced caloric intake. Other interventions, such as TRF and PR or amino acid restriction are somewhat better characterized under isocaloric experimental conditions. In all cases, however, one should carefully evaluate relative caloric intake when considering studies in this area.

KDs

KDs refer to dietary compositions designed to maintain a constant state of ketogenesis, the metabolic production of ketone bodies (acetoacetate, β-hydroxybutyrate, and acetone) as a by-product of fat metabolism in the liver. This results in ketosis, a state of elevated ketone bodies in the blood that can then be taken up and metabolized by other tissues. KDs have been studied in humans for many decades as a treatment for epilepsy and have achieved mainstream popularity because of their palatability and effectiveness at inducing weight loss (*16*). In humans, the most common KD is typically very low in carbohydrates (less than 30 to 50 g per day), with ~75% of calories derived from fats (*17*). Many other KD variations are possible, so long as carbohydrate levels remain low enough to induce ketogenesis, such as the popular high-protein Atkins Diet (*18*). Currently, the long-term health consequences of KDs in humans and the relative merits of low- versus high-protein KD diets are vigorously debated within the nutrition community, with little consensus beyond clear efficacy for epilepsy and weight loss.

KDs recently gained recognition for potential effects on biological aging with two 2017 papers that reported that a low-carbohydrate, low-protein KD is sufficient to increase mean life span as well as health span measures in mice (*19*, *20*). In one study, a 0%-carbohydrate KD that achieved high levels of β-hydroxybutyrate in blood was initiated at 12 months of age and given to the mice either continuously or in a cyclic fashion interspersed with control chow on a weekly basis (*19*). The continuous KD failed to increase life span, whereas the cyclic KD increased mean, but not maximum, life span and improved both memory function and metabolic parameters late in life. In the other study, a low-carbohydrate diet (12% carbohydrates) or a KD (less than 1% carbohydrates) were initiated at 12 months of age (*20*). Both diets appeared to increase median life span relative to control-fed animals, with the KD resulting in a 13% increase in median life span and trend toward a smaller increase in maximum life span, which did not reach statistical significance. Improvements in memory, motor function, and reduced cancer incidence were observed in the mice fed a KD in this study. Both studies observed reduced mTOR activity in the longer-lived mice eating a KD.

One question is whether KD effects are mediated by ketone bodies directly. Ketone bodies produced by the liver enter circulation and are taken up by other tissues, where they can directly enter the tricarboxylic acid cycle through acetyl–coenzyme A, bypassing the need for glycolytic breakdown of glucose. The ketone body β-hydroxybutyrate has also been implicated as a signaling molecule that can act through extracellular receptors to regulate histone acetylation and thereby impact gene expression (*21*). One study reported that β-hydroxybutyrate supplementation could extend life span in *Caenorhabditis elegans* through a mechanism linked to reduced mTOR signaling (*22*), although there is not yet direct evidence

Table 1. Summary of antiaging diets. Life span effect refers to studies in rodents. Number of arrows are intended to indicate relative robustness and consistency of reported effects.

Dietary intervention	Description	Life span effect
Low-calorie interventions		
"Classic" CR	Daily reduction in calories, typically by 20 to 50%, without malnutrition. Macronutrient ratios are unchanged.	↑↑↑
CR without PR	CR in which protein content is modified so that only calories are reduced and protein intake is not changed.	↑↑↑
IF	CR variant with at least 1 day of fasting between feedings. Many classic CR studies used intermittent fasting protocols in which mice were fed three times per week.	↑↑↑
FMD	Cyclic CR in which a low-calorie KD is provided during the restricted phase. In mice, FMD cycles are typically 3 to 4 days followed by 3 days of refeeding.	↑↑
KD	Restriction of carbohydrates to induce ketosis. In mice, carbohydrates are limited to less than 1% of total calories. KDs do not have to be low calorie, but the variations studies in mice resulted in reduced caloric consumption.	↑
Isocaloric diets		
PR	In mice and rats, isocaloric PR has been reported to extend life span, but the effects appear to be much smaller than CR and may be sex-specific in mice.*	↑
Essential amino acid restriction	Restriction of methionine, tryptophan, or BCAA content in the diet. Essential amino acid restriction in mice typically involves reducing methionine by about 80%, tryptophan by about 40%, or BCAAs by about 67%. It remains unclear what extent these interventions share similar mechanisms.	↑
TRF	Ad libitum feeding restricted to a specific period of the day. In people, a common TRF protocol is 16:8 (hours fasting: hours feeding). In mice 12:12 has been tested.†	↑
Isocaloric IF	IF where the IF group consumes an equal number of calories as the control group by overfeeding during the *ad libitum* phase.‡	↑

*A recent report found that PR increased life span in male mice but not female mice (*55*). the experimental mice consumed slightly less kcal/day than did the control mice (*40*). conditions (*92*).

†One study of only male mice reported 11% life span extension in "isocaloric" TRF mice, but

‡Every-other-day feeding increased life span in one study by 13% under roughly isocaloric

that ketone body metabolism is sufficient to confer increased life span in mice. Ketone esters are reported to reduce anxiety-like behaviors and decrease amyloid-β and amyloid-τ deposits in a mouse Alzheimer's disease model (*23*) and have also been reported to reduce blood insulin and glucose levels and to inhibit mTOR signaling (*24*). Taken together, these findings are highly suggestive that ketone esters themselves could have antiaging properties and highlight the importance of additional research in this area.

IF and FMDs

Fasting has long been touted for its putative health benefits in different cultures, and recent years have seen a resurgence of research on possible antiaging effects of diets that incorporate fasting or "fasting-mimicking" components. Reviews of this topic will often make blanket statements about the health benefits of IF for numerous age-related conditions in mice, and although there is certainly evidence to support such assertions, the experimental protocols used generally amount to CR (*25*, *26*); it appears that modern IF protocols are largely a rebranding of classic CR methods [for example, (*3*), where the CR mice were fed three times per week]. Hungry laboratory mice (and any CR researcher will tell you that their mice are hungry) will typically eat all of the available food in their cage quickly compared with control-fed mice. Thus, even in a CR study in which mice are fed daily, the restricted mice will typically be fasting for at least 18 hours between meals. This is not intended to downplay the potential importance of the physiological changes associated with fasting, such as ketogenesis, but is simply an observation that the majority of preclinical studies referring to fasting-based experimental protocols cannot be differentiated from CR because researchers rarely ensure isocaloric conditions (Table 1).

Over the past decade, FMDs have emerged as a highly studied cyclic variant of CR designed to induce metabolic responses akin to fasting through a nutrient-dense, low-calorie diet. FMDs induce ketogenesis by restricting protein and simple carbohydrates while maintaining high fat levels (*27*, *28*) and could therefore be considered as an intermittent KD. Initial studies in rodents showed that bimonthly 4-day FMD reduces both body and organ size and improves a wide range of age-related parameters, including adiposity, tumor burden, motor and cognitive function, neurogenesis, and median (but not maximum) life span (*27*). One interesting effect of cyclic FMD was the induction of atrophy and quiescence followed by vigorous regeneration and stem cell activation in several tissues. Similarly, metabolic parameters such as blood glucose, insulin, IGF-1, and IGF-1BP were reduced during the FMD phase and returned to control levels during a regular feeding regimen in both mice and human subjects (*27*, *28*).

There is substantial clinical interest in FMDs based on the rationale that their cyclic nature will increase compliance relative to traditional diets. In one randomized controlled crossover study, trimonthly FMD cycles of 5 days each reduced body mass index (BMI), fasting blood glucose, and blood pressure in obese, prediabetic subjects and subjects with hypertension, respectively (*28*). Cycles of FMD appear potentially beneficial in other clinical contexts, such as multiple sclerosis, autoimmune diseases, and cancer (*29*–*31*). Use of FMDs to improve outcomes in combination with chemotherapy is a particularly active area of research, with initial studies indicating that FMDs increase tumor sensitivity to chemotherapy in mouse models of breast cancer and melanoma while reducing collateral toxicity to healthy cells (*30*, *31*). However, a recent clinical trial failed to detect any difference in the specificity of chemotherapy in breast cancer patients undergoing FMD cycles (*32*), possibly because of low compliance (*33*).

Like IF, most FMD studies have been performed under conditions in which the experimental group consumed fewer calories than did the control group, and the extent to which isocaloric IF or FMDs have a substantial impact on life span or age-related pathology remains unclear. Some FMD studies report that FMD and control mice consume energetically equivalent amounts of food when normalized to body weight (*27*), but because the FMD mice weigh less than control mice, their total caloric intake is likely reduced. There is evidence that true isocaloric IF implemented as alternating feeding and fasting days (1:1 IF) is sufficient to induce ketogenesis during the fasting day and may improve metabolic homeostasis, stress resistance, and markers of inflammation compared with daily pair-fed mice (*34*). A 2-day feeding/1-day fasting isocaloric regimen (2:1 IF) also prevented weight and fat gain, maintained glucose and insulin homeostasis, and reduced adipocyte hypertrophy in mice fed an obesogenic diet, despite comparable energy intake. However, a recent 1:1 IF study over 3 weeks in lean, healthy people found that IF was less effective than isocaloric daily energy restriction and showed no benefits for measures of metabolic regulation or cardiovascular health (*35*). Because all of these studies were limited in scope and duration, future studies will need to assess whether isocaloric IF or FMDs have substantial long-term benefits on health and longevity in either rodents or people.

TRF

TRF can be considered as a variant of IF in which subjects receive food every day but only for a specified time window. Isocaloric TRF studies in rodents suggest improvements in several metabolic parameters, including glucose and insulin homeostasis, energy expenditure, liver pathology, and resistance to different obesogenic diets (*36*–*38*). Intriguingly, isocaloric TRF seems to promote and maintain intrinsic circadian rhythms in mice (*36*, *37*), a phenotype also associated with CR (*39*). To the best of our knowledge, only one study has attempted to carefully examine the effect of isocaloric TRF on life span and age-related health outcomes in mice (*40*). In that report, which was limited to male mice, the TRF animals were trained to eat all of their food in a 12-hour window each day. The TRF mice were fed a diet intended to be isocaloric to the ad libitum group; however, the TRF animals still ended up eating less than did the ad libitum–fed animals. A 30% CR group in which the mice ate all of their food in a 3-hour window each day was also studied in parallel. The TRF group lived about 11% longer than did the ad libitum group, on average, whereas the 30% CR group showed a 28% increase in mean life span. Circulating levels of β-hydroxybutyrate were higher in the CR group but not in the TRF group.

Despite promising results of TRF in animal models, human studies are mixed. Some studies show only mild improvements (*41*), even when subjects naturally restricted themselves to 75 to 80% of their daily intake during feeding (*42*). Other studies indicate detrimental effects on glucose homeostasis (*43*). These studies designed their feeding window without regard for circadian variation. Larger, longer-term studies, carefully crafted around human circadian rhythms, are needed to determine whether TRF regimens can be beneficial to metabolic homeostasis and ultimately aging in humans.

PR and amino acid restriction

The importance of protein as a dietary modulator of life span can be traced back to work in the 1920s that demonstrated that trout raised on a protein-deficient diet were both developmentally delayed and longer-lived (*44*). A few years later, dietary PR was found to delay development, sexual maturation, and signs of aging in rats (*45*). Since these early reports, numerous studies have described increased life span and reduced age-related pathology resulting from PR in rodents, which has been proposed to be mediated largely through reduced growth hormone, IGF, and mTOR signaling (*46*, *47*).

As with IF and FMDs, a particularly challenging aspect of the literature related to PR is disentangling the effects of reduced caloric intake from specific effects of protein itself as a dietary macronutrient. Speakman and colleagues performed a detailed meta-analysis comparing published effects of dietary interventions on life span in mice and rats and

concluded that although there is evidence for life span extension from PR alone, the effects are substantially reduced compared with those reported associated with CR (*13*). This is consistent with work from Masoro and colleagues, who compared isocaloric PR to classic CR in rats and found that PR alone increased median life span by about 15% compared with about 50% increase from CR (*48*).

Nutritional geometry studies, carried out under ad libitum conditions in which ratios of different macronutrients are varied across numerous diets, provide an independent line of evidence that dietary protein may have an outsized impact on longevity in insects and mice, relative to other macronutrients (*49*). In mice, for example, a comprehensive study of longevity effects across 25 different diets found that those with lower protein-to-carbohydrate ratios yield the longest maximum life spans (*50*). The authors concluded that longevity and health are optimized in mice when protein is replaced with carbohydrate. However, the absolute life spans of the mice in this study were generally lower than those reported elsewhere for the same strain background (C57BL/6J), and the lowest-energy diets failed to yield the longest life spans (*50*). Further, out of all the diets studied, the one that resulted in the longest median life span (139 weeks) was quite high in protein (42% protein, 29% carbs, and 29% fat). Thus, the relationship between dietary protein and longevity, at least under ad libitum conditions, appears to be quite complex and influenced both by other macronutrients and additional variables that are not yet understood.

In addition to reducing the availability of total dietary protein, there are several reports of life span extension from restriction of specific essential amino acids, which must come from the diet and cannot be synthesized endogenously. The earliest of these may be from Segall, who published in 1977 that restriction of dietary tryptophan delayed growth, reduced cancer, and increased life span in rats (*51*). Orentreich and colleagues later found that restriction of dietary methionine (or more properly, sulfur amino acids that include both cysteine and methionine) similarly increased life span in rats (*52*). These seminal discoveries went largely unappreciated for many years but have gained attention as others reproduced and extended these findings to yeast, worms, fruit flies, and mice (*53*). More recently, restriction of the dietary BCAAs leucine, valine, and isoleucine has also been found to increase life span and delay age-related frailty in both fruit flies (*54*) and mice (*55*). BCAA restriction appears to increase life span through the inhibition of mTOR signaling. Other studies show that restricting methionine and cysteine is key to promoting a protective DR-mediated stress response that is blocked with mTOR activation. This sulfur amino acid restriction appears to be mediated through a mechanism that involves increased production of hydrogen sulfide gas through the transsulfuration pathway (*56*). Unlike most of the other antiaging diets that include CR, mice restricted for particular amino acids appear to actually eat more food than control-fed animals but fail to gain weight (*57*, *58*).

Along with inhibition of mTOR, the hormone fibroblast growth factor 21 (FGF21) has emerged as an important mediator of longevity benefits from PR and perhaps also amino acid restriction. FGF21 is actively secreted in response to reduced dietary protein in both mice and humans and is required for metabolic improvements in response to PR (*59*). FGF21 overexpression in mice fed ad libitum is sufficient to robustly increase life span (*60*). There is also evidence that FGF21 can be similarly induced through methionine restriction (*61*) and KDs (*62*). It has been proposed that FGF21 modulates life span in mice primarily by reducing growth hormone and IGF-1 signaling in liver (*60*).

Do antiaging diets work in the "real world"?

Fad diets spawned from legitimate scientific research are nothing new, and recent years have seen a notable infiltration of antiaging diets into mainstream society. The renowned CR researcher Roy Walford attempted to popularize CR in the 1980s with his book *The 120 Year Diet: How to Double Your Vital Years* (*63*). Walford's "CR Society" never grew beyond a small group of followers, likely because of the severe abstinence required to maintain a CR lifestyle, and Walford himself died at the age of 79, well short of the 120 years promised in the book title. Recently, however, several less stringent variations on CR have achieved greater popularity, including IF, TRF, PR, and KDs. Some researchers studying these nutritional interventions follow in Walford's footsteps by practicing various forms of CR themselves and making dietary recommendations to the general public. Absent results from carefully designed clinical trials, this raises thorny questions around safety, efficacy, and scientific integrity.

When considering the evidence for antiaging diets in humans, we address two primary considerations. First, how strong is the case that these interventions actually slow or reverse biological aging in people? Second, are there potential side effects that may offset any benefits for healthy longevity? Before delving into these topics, however, we must differentiate between health benefits from modulation of biological aging versus effects that derive from being anti-obesogenic. CR, PR, and KDs are each used clinically to induce weight loss (*64*), and there is no question that weight loss in obese individuals can reduce disease risk. One rationale in favor of recommending these diets to the general public is the perception that they are all likely healthier, at least in the short term, than the typical Western diet. These potential benefits in overweight individuals should not, however, be conflated with effects on biological aging.

Unfortunately, it is not currently possible to know whether CR-like diets affect biological aging in people (Box 2). Unlike mice, controlled studies would need to be performed over many years to assess long-term benefits for life span and health span in humans. The recent development of various "aging clocks" that accurately predict chronological age, and may soon be useful for predicting biological age, offer the possibility that this question may be addressable in the relatively near future (*65*). For now, however, the data remain correlative. One line of evidence often cited to support antiaging effects of CR in natural human populations comes from studies of Okinawans, who inhabit a small Japanese island and smaller islands in the surrounding archipelago where the indigenous population historically consumed about 20% fewer calories than did the population of mainland Japan. Traditional Okinawan diets are very low protein (9% of total calories) and high carbohydrate (85% of total calories) (*66*). Historically, Okinawans enjoyed the longest life expectancy at birth and highest centenarian prevalence in the world, with remarkably low rates of age-associated diseases, such as cancer, heart and cardiovascular disease, and diabetes (*67*). Another line of evidence for health benefits from CR comes from the Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy (CALERIE) studies. These are a series of controlled clinical trials in normal and overweight adults subjected to a 25% reduction in caloric intake over periods ranging from a few months to 2 years. The results of these studies were generally consistent with improved clinical biomarkers of health such as decreased weight, enhanced insulin sensitivity and glucose tolerance, and improvements in major cardiometabolic risk factors (*68*). The CALERIE data are further complemented by uncontrolled studies of people self-practicing CR. Data from individuals self-practicing CR are also consistent with improved age-related health measures, including reduced weight and fat mass, lower blood pressure and other markers of heart disease, and improved glucose tolerance and insulin action (*69*, *70*).

Despite these suggestive data, there are concerns that laboratory nutrition studies may introduce artifacts that limit translation to humans. One example of this is that laboratory strains of rodents have been subjected to strong selection for rapid growth and early reproduction, which may lead to sensitization to the life span–extending effects of CR on

Box 2. Common fictions about antiaging diets.

Fiction: CR always "works." Although there are many reports of life span and health span extension from CR, there are also multiple published examples in which CR failed to extend life span. Among these are studies of wild-derived mice (*71*) and studies of genetically inbred mouse strains (*72*). Of the two long-term studies in rhesus monkeys, one reported a substantial increase in life span, whereas the other failed to detect any significant change (*98*, *99*). Although negative results can be challenging to interpret, the efficacy of CR even in laboratory animals appears to be highly dependent on sex, genetic background, the level of restriction used, and other variables yet to be identified (Fig. 1).

Fiction: CR extends life span only by preventing cancer. Although CR has been shown in many studies to have potent anticancer effects in rodents, it also delays age-related declines in immune, brain, heart, muscle, kidney, reproductive, and other tissues (*5*). CR also extends life span in nonmammalian species that do not get cancer, such as budding yeast, fruit flies, and nematode worms (*6*).

Fiction: Individual macronutrients are "good" or "bad" for aging. Dietary composition, total caloric intake, and feeding interval all have the potential to affect longevity and health span. It is possible to extend life span in mice by limiting total caloric intake, limiting primarily carbohydrates, or limiting primarily protein or even specific amino acids (Table 1). The mechanisms underlying these effects are complex and still poorly understood, even in highly controlled environments.

Fiction: Antiaging diets are known to slow aging in people. Despite their recent popularization, there is not yet strong evidence that any of the antiaging diets studied in laboratory animals have substantial long-term health benefits in nonobese humans.

growth-promoting signaling pathways such as growth hormone and mTOR (*71*). Another concern is that features of the laboratory environment may affect how animals respond to CR-like diets. Among these, mice and rats are typically housed in specific pathogen-free conditions and receive daily supervision and veterinary care as needed. Because of small body size and low temperatures at which they are housed, mice expend upward of half of the energy they consume just to maintain core body temperature (*72*). They experience consistent light-dark cycles and consume a refined, fixed composition chow for most of their adult life. None of these things are true in people, and it seems plausible that human environmental variation could have a large impact on health outcomes from dietary intervention. For example, severe CR can impair both immune function and wound healing (*73*), which could offset any potential life span–extending benefits under adverse environmental conditions in which the immune system is challenged (for example, a global viral pandemic) or in the absence of quality health care. Further complicating this interaction is the large variety in nutritional quality of human dietary composition, even among individuals who are consuming comparable total calories or macronutrient ratios.

The impact of genetic background may also limit translation from laboratory models to humans. Although not widely appreciated, genetic variation plays a large role in determining individual responses to CR in laboratory animals (Fig. 1). In one study, for example, a 40% reduction in caloric intake among recombinant inbred mouse lines significantly shortened life span in a greater number of genetic backgrounds than it extended (*74*). Sex-specific differences were also apparent, with CR having opposite effects on life span between sexes in some strains. Although this particular study was underpowered at the individual genotype level (*74*), several others have reported large variation in the response to CR in individual mouse and rat strains (*75*), and very similar genotype-dependent effects of DR by glucose restriction have been observed in budding yeast (*76*). The role of genetic variation in efficacy of other antiaging diets is largely unexplored, but one recent study found that about one-third of fruit fly strains experienced life span shortening in response to PR (*77*). It seems clear that a more detailed understanding of the mechanisms underlying variable response to antiaging diets in laboratory and genetically diverse animals will be important for predicting both efficacy and risk in people.

Differences in life span and age-associated nutritional requirements between humans and laboratory rodents present yet another layer of complexity when considering translation of antiaging diets. Although most animal studies examine the effects of lifelong nutritional intervention, very few people will maintain a CR (or PR or KD) lifestyle continuously over many decades of adulthood. Instead, repeated cycles of ad libitum and CR consumption is the norm. Detrimental effects of so-called "yo-yo dieting" are well documented and, taken to extremes, can result in a potentially fatal refeeding syndrome with severe consequences, such as hypotension, kidney injury, and heart failure (*78*). Even moderate dietary interventions are not without some level of risk over the long term. For example, one study found that low-protein diets are associated with reduced mortality in young people but higher mortality in people over the age of 65 years (*79*).

These correlative observations should provide a cautionary note. Although many people tend to assume that dietary interventions are safe, the biological effects of these antiaging diets are profound and generally less specific than pharmacological interventions. Like any drug, dietary interventions have a dose-

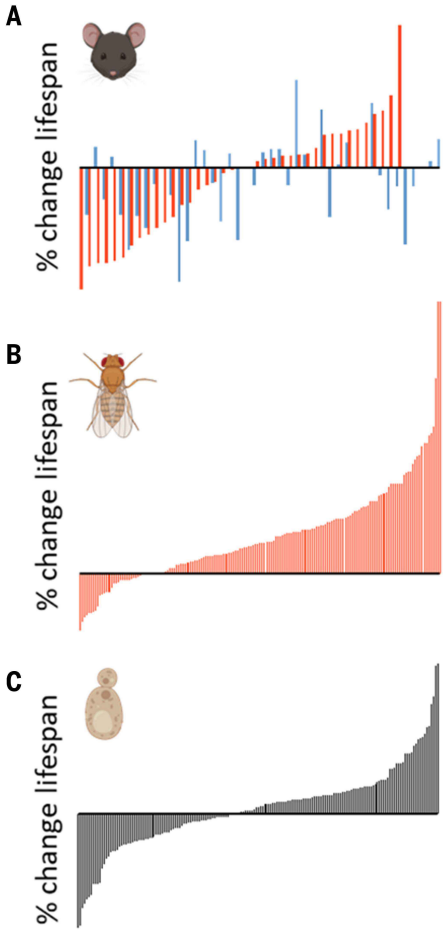

Fig. 1. Percent life span change on restricted compared with unrestricted diet for genetically distinct strains of model organisms. In every case, each bar along the *x* axis represents a different genetic background, and the percent change in life span of that strain in response to dietary restriction is shown on the *y* axis. (**A**) Change in mean life span for recombinant-inbred female (red) and male (blue) mice under ad libitum and 40% CR diets (*74*). (**B**) Change in median life span in genetically variable female flies from a natural population under a 10-fold PR (*77*). (**C**) Change in mean life span for single-gene-deletion mutant yeast under a 40-fold glucose restriction (*76*). Organism cartoons were created with BioRender (https://biorender.com).

response profile and at high enough "doses" will lead to substantial adverse effects and ultimately death. Among the potential side effects of CR-like diets are poor thermotolerance, loss of libido and sexual dysfunction, psychological problems, chronic fatigue, poor sleep, muscle weakness, susceptibility to infection, impaired wound healing, and social isolation (*80*). There is a very real likelihood that any given CR-like diet could enhance longevity in some people while shortening life span in others. The optimal nutritional strategy for longevity will certainly be different in different people, and there are few studies that quantify short-term or long-term side effects of antiaging diets in adults.

mTOR inhibition: A common mechanism for antiaging diets?

Although many questions remain, research on antiaging diets has had a large impact on the field by providing insight into fundamental mechanisms of aging. The physiological consequences of dietary interventions are complex and multifactorial, even in relatively simple laboratory model organisms. Despite this, intriguing similarities across the spectrum of antiaging diets in diverse model systems have emerged that suggest at least partially overlapping mechanisms of action. As alluded to above, these mechanisms appear to converge on key nodes in a highly conserved aging-regulatory network (*7*, *8*). Although several components of this network have been implicated in mediating aspects of CR (*10*), a case can be made that mTOR is a particularly relevant and robust molecular transducer of diet-induced antiaging signals (*81*).

The mTOR protein is a kinase that mediates nutrient response signaling by acting in two distinct complexes, mTORC1 and mTORC2. Both complexes are affected by nutrient and growth cues, but mTORC1 has received the most scrutiny owing to observations that its inhibition is sufficient to mimic the effects of CR on life span in yeast, worms, flies, and mice (*82*). This has been replicated both genetically and pharmacologically (rapamycin) in each of these model systems. Like CR, mTORC1 inhibition appears to broadly delay or reverse age-related phenotypes across multiple tissues in mice, including the brain, heart, liver, kidney, immune system, skeletal muscle, auditory system, adipose, ovaries, and the oral cavity (*82*–*84*).

There is accumulating evidence that each of the antiaging diets discussed here can inhibit mTORC1 signaling through both direct and indirect mechanisms (Fig. 2). Specifically, mTORC1 is directly activated by leucine, an effect mediated in part by the sestrin family of proteins that inhibit mTORC1 only when not bound to leucine (*85*). Glucocorticoid signaling, which is induced by CR (*86*), also has an inhibitory effect on mTORC1 (*87*). mTORC1 is activated by the growth-promoting hormone IGF-1 through Akt and the tuberous sclerosis complex (TSC), and reduced IGF-1 signaling is one of the classic hallmarks of CR and CR-like diets (*5*). AMPK becomes activated in response to CR and regulates mTORC1 through phosphorylation of TSC (*81*). Several processes are proposed to mediate the life span–extending effects of CR downstream of mTORC1 inhibition. Covered in detail elsewhere, these include activation of autophagy, inhibition of mRNA translation, enhanced mitochondrial function, increased ketogenesis, improved stem cell function, and attenuation of senescence-associated inflammation (*81*, *88*, *89*).

We recognize that many questions remain regarding the relative importance of mTORC1 in mediating effects of CR and other antiaging interventions, and others have suggested that other factors such as sirtuins and AMPK are equally important (*90*). As described above, we favor the model that these factors all act together within a complex network that has yet to be fully characterized. Our focus here on mTORC1 reflects that it appears to be a particularly useful node within this network for modulating aging, as evidenced by the robust and reproducible data that support beneficial effects on life span and health span from pharmacological or genetic inhibition in yeast, worms, flies, and mice (*81*, *82*). We recognize that mTORC1 regulation is extremely complex, with tissue-dependent and circadian components that are still poorly understood. It is also clear that CR and mTOR inhibition have overlapping but distinct physiological effects so that treatment with rapamycin, for example, does not recapitulate all of the effects of CR, and vice versa (*91*). We are not aware of genetic backgrounds that fail to show life span extension in response to mTORC1 inhibition in mice, and unlike the case for CR, there have been no studies yet broadly assessing this question. High doses of rapamycin used to treat organ transplant patients are associated with multiple side effects, and although side effects are greatly reduced at lower doses in healthy people, whether mTORC1 inhibition is a useful therapeutic strategy to combat aging in humans remains unclear. Undoubtedly, much remains to be understood regarding the various interactions between dietary nutrients, longevity pathways, and healthy aging.

Conclusion

Research on antiaging dietary interventions that increase life span and health span in laboratory models has greatly facilitated our mechanistic understanding of biological aging. Evolutionarily conserved signaling pathways

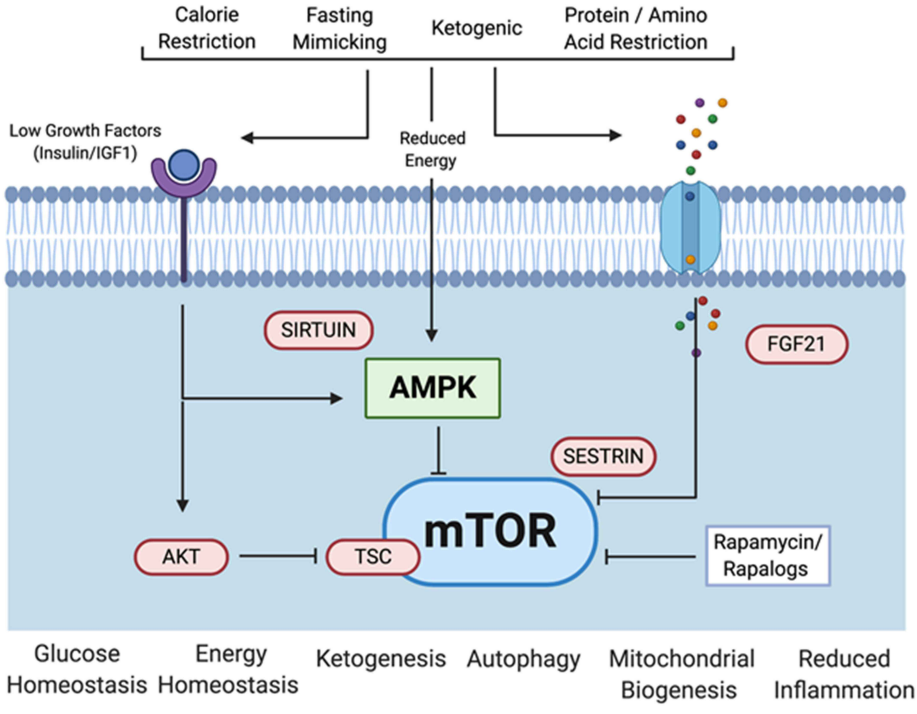

Fig. 2. Diet modalities, molecular mechanisms, and downstream consequences of antiaging diets. Dietary interventions that affect aging in mice limit one or more of the major dietary macromolecules and elicit cellular responses through a complex nutrient-sensing network. Key components of this network that have been implicated in effects on life span and health span in various laboratory model organisms include mTOR, FGF21, AMPK, insulin and IGF-1 receptors, AK strain transforming (AKT), sestrin, and sirtuins. The figure was created with BioRender (https://biorender.com).

appear to mediate many of the overlapping effects of antiaging diets, and their study has provided molecular targets for pharmacological interventions that may prove useful for increasing healthy longevity and reducing disease burden in humans. Although these diets have already achieved mainstream popularity in some cases, many questions remain about individual outcomes and relative risks associated with their long-term implementation. Future research should focus both on better understanding the cellular and molecular mediators of antiaging diets under highly controlled laboratory conditions as well as the impact of genetic and environmental variation on health outcomes associated with these diets.

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Antiaging diets: Separating fact from fiction

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Caution around the fountain of youth

The scientific and popular literature is full of claims for diets that delay or reverse the aging process (at least in model organisms). But how do these interventions work? Is it the amount of food, the timing of food intake, the proportion of certain macronutrients? In a Review, Lee *et al.* explore the fact and fiction of dietary prescriptions for a healthier and longer life. They propose that one unifying concept may be convergence on the signaling pathway mediated by the protein kinase mTOR (mechanistic target of rapamycin). Another conclusion is that the efficacy and safety of these diets for humans largely remain to be established. —LBR