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Perspective

Biological Approaches to Mechanistically Understand the Healthy Life Span Extension Achieved by Calorie Restriction and Modulation of Hormones

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Calorie restriction and reduced somatotropic (growth hormone and insulin-like growth factor-1) signaling have a widespread though not universal ability to extend life. These interventions are considered central tools to understanding the downstream events that lead to the increase in healthy life span. As these approaches have been validated, the animals phenotyped, and the mechanisms proposed, many challenges have emerged. In this article, we give several examples and propose several considerations, opportunities, and approaches that may identify major mechanisms through which these interventions exert their effects, and which may lead to drug therapy to increase "health span."

Key Words: Healthylife span—Caloric restriction—Growth hormone—Insolin-Like growth fact.

CALORIE RESTRICTION: THE PROMISE

Demographers have observed in several human populations that the environment can have a major effect on life span. In the latter part of the 20th century, cigarette smoking was the major risk for environmentally related death in the United States. Currently, it is the epidemic of obesity, suggesting that calorie intake contributes to human aging and life span. Interestingly, from the perspective of the biology of aging, experiments involving calorie restriction (CR) in rodents in 1935 provided the first promise for modulation of life span. Several developments have made CR central in understanding the biology of aging.

1. CR experiments have been validated in rodents generally available for research, and the approach has been repeatedly reliable. Dose–response studies of CR have shown beneficial effects of various levels of CR, although at a certain point, severe CR will result in death.
2. CR experiments have been done in other mammalian species including dogs and rabbits, and the results indicate that the action of CR is universal in extending life span in most mammals. Preliminary results of CR studies in rhesus monkeys indicate that this intervention can also increase longevity.
3. Many experiments have indicated that the relative proportions of macronutrients (ie, carbohydrates, fats, or proteins) are not important but rather the total decrease in calories determines the effect on longevity (thus termed CR rather than dietary restriction). However, this issue has been recently reopened.
4. Calorie-restricted animals seem robust until late ages (ie, they appear to have a longer "health span"), and the most consistent physiological effects of CR are reduced body weight and temperature. In cross-sectional studies of pathology, CR animals at the same age have less age-related pathology (mainly tumors), and although eventually tend to die with similar pathology more cases of unknown deaths, suggesting a delay in the rate of aging.
5. Biologic characteristics of CR animals have shown numerous changes in the transciptome, metabolome, and characteristic plasma protein profiles, including increase in stress hormones (mainly corticosterone or cortisol depending on the species). They have also shown declines in the growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis, insulin (and glucose), thyroid hormone, and reproductive hormones. These findings provide support for various theories of aging such as stress as a modulator, decreased metabolic rate enhancing life span, a trade-off between longevity and reproduction, and evidence that the insulin/IGF-1 signaling pathway is a regulator of aging.
6. More recently, the effects of CR on longevity have been shown in many taxonomically distant organisms including yeast, nematodes, and flies, suggesting its universality.

CR: THE CHALLENGES

The goal of ongoing studies is to identify pathways that can be modulated and lead to healthy life span in humans.

Although many studies have continued to shed light on the biology of CR, other recent work has challenged this paradigm. However, those results certainly do not undermine the use of this approach, but as will be discussed later, they change some of the interpretations.

1. A reductionist approach in studying CR is recapturing each of its major phenotypic characteristics in genetic models and testing if they affect longevity. Excluding the example of lowering IGF-1 action (see below), several models have resulted in negative results. For example, increasing corticosterone in transgenic animals (1) or lowering insulin and glucose in a glucose transporter 4–overexpressing transgenic animals (2) has not changed their life span. Another interesting fact is that most calorimimetic agents that have been tested had toxic effects and reduced life span (3). The questions raised by these experiments include whether the relevant functions need to be measured more downstream, or if the numerous changes in the phenotype of CR animals should be established more "upstream," closer to the nutrient-sensing pathways, to move the field forward (see below).

2. CR does not enhance longevity in all strains of laboratory animals or all species (4). Experiments in recombinant inbred strains that have not been previously studied have demonstrated that although many of them have extended their life span by CR, in many others longevity was unchanged or even decreased (B. Rikke, T. Johnson, & J. Nelson, PhD, unpublished, 2008). An obvious possibility is that for some of these strains, CR has been insufficient or excessive, and perhaps a dose–response study would have revealed this problem. Nonetheless, it challenges the universality or the limits of the CR "model" in mammals.

3. The employment of CR in invertebrates is problematic and may not always be a real restriction of calories. For example, yeasts are considered calorie restricted when they are grown in 100 mg/dL of glucose rather than in 300 mg/dL. This does not indicate the actual flux of glucose and may affect life through mechanisms of glucose toxicity. Although some isotopic technologies have been developed in other models, they have not been sufficiently validated. To discover the key mechanisms by which CR delays aging, studies in species, strains, or mutants in which CR is not effective are likely to be informative.

4. Although the CR paradigm has worked in the laboratory, it involves several restrictions that may make its applicability to humans somewhat limited. For example, most animal facilities at our universities are specific pathogen–free, and it is not clear if CR also protects against infection, a major killer of the elderly. This is particularly important because adipose tissue is part of our innate immune system, and a significant reduction in adipose tissue may jeopardize this immunity. Moreover, rodents do not seem to die from the same causes as humans, and the relevance to many age-related diseases in humans has not been tested in partially humanized rodents (eg, Low Density Lipoprotein (LDL) receptor or Apolipoprotein E (APOE) knockouts for cardiovascular disease).

5. Rodents in the wild are fairly lean and may run several miles a day in their pursuit of food. Thus, in experiments using caged rodents that are allowed to eat ad libitum, it is not entirely clear whether longevity benefits are attributable to CR as it prevents obesity (compared with the levels in the wild) or to the alteration in stress response, reproduction, and so forth. For example, a recent experiment demonstrated that removal of visceral adipose tissue (the "bad" fat) in ad libitum–fed animals also resulted in life extension, suggesting that obesity may be relevant to CR (5). Similarly, the FIRKO mice (knockout of insulin receptor in adipose tissue) have a depletion of adipose tissue and live longer (6). This is important because in human populations, being too lean has been repeatedly shown to be associated with increased mortality, suggesting that excessive CR would be deleterious in humans.

6. Even if CR is beneficial in all animal models, it obviously needs to be relevant to humans. Some argue that CR may not work in humans because the ratio between CR needed to affect reproduction and produce metabolic deficit cannot be practically achieved (4). Ongoing CR studies in nonhuman primates have yet to produce conclusive evidence for life span extension, excluding the example of diabetes, which typically reacts to weight loss in humans. It is important to note that these studies have not been completed, but bigger effects have been anticipated based on rodent studies. An National Institute of Aging (NIA) effort to induce CR in middle-aged overweight humans has been initiated, but thus far has involved only a short period of CR. These studies have generally recapitulated the phenotype seen in other species, except for lack of decreased plasma IGF-1 levels. A group of humans voluntarily committed to CR (7) is being followed and has shown major cardioprotective benefit, but no data on the incidence of diseases or on life span have been generated, and as emphasized before, this is an important consideration, as extreme leanness identified as a mortality risk in humans.

Future studies will determine how responses to CR differ from responses to starvation and whether CR activates a "survival program" in primates as it does in rodents and invertebrates.

CR: The Opportunities

Some interventional and research opportunities derive directly from the promise and challenges mentioned above, whereas some reflect development in technologies and other disciplines.

1. To avoid a reductionist approach, it may be more biologically sound to discover the nutrient sensors and the upstream effectors leading to the life-extending effects of CR. There are a large number of nutrient sensors being proposed, but three in particular have been directly implicated with aging. The most exciting example is the role of sirtuins in sensing the metabolic state of a cell by Nicotinamide Adenine Dinucleotide (NAD) balance. The mammalian sirtuin, sirt1, is upregulated by CR and its main (but not singular) biologic action is deacetylation of histones. The activation of sirt1 affects a variety of physiological parameters relevant to aging. The red wine component resveratrol is a sirt1 activator and has been shown to extend life span in high fat–fed animals (8) and to affect health span in animals fed a regular diet, although in this model, resveratrol treatment did not result in increased longevity (9). A second nutrient sensor potentially affecting life span is the mammalian target of rapamycin (mTOR), a regulatory pathway that is linked to other metabolic pathways, particularly insulin signaling (10). For example, mTOR has been implicated in the in vivo sensing of amino acids in the mammalian brain, causing the regulation of peripheral metabolism (11). Genetic modulation of mTOR in invertebrates and modulation of mTOR in mammals with the antagonist rapamycin have further implicated its role in the chronic and acute sensing of nutrients. Finally, the hexosamine biosynthesis pathway (HBP) is a central nutrient-sensing mechanism. Increased glucose and Free Fatty Acios (FFA) (through increased fructose-6-phosphate) and the amino acid glutamine (through fructose-6-phosphate amidotransferase) increase the flux through this pathway by several fold and result in the O-glycosylation of intracellular proteins. This glycosylation pathway activates transcription factors (eg, SP1) that are involved in the nutrient-mediated modulation of the expression of numerous proteins. Activation of HBP induces insulin resistance and regulates the secretion of several cytokines and thrombotic factors in young rodents but more severely so in old rodents (12). Although these examples probably represent major nutrient-sensing pathways, they do not include all examples worth pursuing. The challenge is to demonstrate the relevance of a nutrient-sensing pathway to aging, CR, and life span.

2. A high-throughput reductionist approach to identify signaling pathways relevant to CR can be achieved in invertebrates by screening and other genetic tools to detect mutations or gene silencing that imitates CR. Such an approach has been used to identify long-lived mutants, epistatic pathways, and networks, and though expensive, it may ultimately prove cost-effective. The challenge would be to apply these approaches to mammals. As suggested earlier, genetic analysis could be applied to any experimental model that was shown to conclusively reflect true CR.

3. The identification of specific targets is obviously critical for development of pharmacological CR mimetics. Approaches to identify and implicate nutrient sensing have to examine cell-autonomous, organ-specific, or whole-body effects. For example, recent evidence suggests that nutrient sensing can be detected in neurons of nematodes and not necessarily in other cells. In fact, the mammalian hypothalamus senses both acutely (eg, insulin increases in response to carbohydrates) and chronically (eg, leptin derived from adipose tissue) the nutritional status of the body. Leptin's action fails with aging, implicating it in some of the metabolic alterations in response to CR. Recently, in vivo studies in mammals have demonstrated how the brain (mainly the hypothalamus) senses directly the nutrients glucose, free fatty acids, and amino acids and regulates endogenous energy production by the liver (11,13,14). As mentioned earlier, another example for a major site for nutrient sensing is the adipose tissue. Adipose cells and adipose tissue macrophages produce fat-derived peptides, inflammatory cytokines, and thrombotic factors that are relevant to aging, CR, and life span. Thus, an organ-specific approach in the studies of the effects of CR is of great value and relevance to the understanding of potential CR effects in the human.

4. The challenges and opportunities stated above underline the complexity of the aging system and the fact that hundreds of factors and numerous pathways in all organs are involved in the response to CR. It is therefore crucial for the field of biogerontology to embrace a systems biology approach (see article by West and Bergman, [15]). For example, system biologists can identify different elements that the various CR models coexpress across cells and organs, time, and environmental conditions. Most importantly, they can help identify which of the genetic elements or systems will provide the best explanation for the phenotype observed.

5. Descriptive studies can also be crucial to further progress of research on CR. First, various tools can be applied to describe the clinical phenotype of the model, its strength, robustness, cognitive function, and so forth, which are indicative of healthy life span. Second, a metabolic assessment is important to describe basic and stimulated metabolic fluxes and to characterize energy metabolism. Third, it would be wonderful to have a biomarker imprint for CR and aging. For practical reasons, markers of health span could perhaps negate the need to perform long and expensive longevity studies, but this has proven to be very challenging.

IGF-1 ACTION: THE PROMISE

One of the most conserved pathways that affect longevity in invertebrates (knockdown of the IGF-1/insulin signaling pathway) and mammals (GH-deficient Ames and Snell dwarf mice, GH-resistant GH receptor knockouts, IGF-1 receptor

heterozygotes, etc.) can be linked to decreased IGF-1 action (16–19). Importantly, both GH-deficient and GH-resistant mouse mutants have many symptoms of delayed aging (17,20). Recent reports suggest that centenarians have functionally significant IGF-1 receptor polymorphisms and mutations compared with the controls, suggesting involvement of this pathway in the control of aging in humans (21).

An intriguing aspect of studying long-lived mutants is that their cells are resistant to a variety of stressors and toxins (22) (see also article by Miller, [23]). Although GH/IGF-1 are hormones with known receptor-mediated functions in most tissues, studies in cultured cells support a hypothesis that low IGF-1 action has intrinsic cellular effects that may be very important in determining the phenotype of aging.

IGF-1 Action: Challenges and Opportunities

As is the case for CR, several results have challenged various aspects of the suggested role of IGF-1 in understanding the biology of aging and translating the data to human aging.

1. One limitation of the mammalian mutants used in this research is that the genetic alteration can affect development beginning after conception and continuing through intrauterine growth and puberty, which may "imprint" their effects onto later life. For example, the resistance of fibroblasts of dwarf mice to external stressors and toxins begins in the first few weeks of life, suggesting early possibly epigenetic effects. If indeed the major role is in early development, it may not be feasible to translate these findings into devising human interventions. Another limitation of some of the long-lived mouse mutants is their deficiency of several other hormones including thyroid and thyroid-stimulating hormones that make those robust models complex. Efforts to ablate GH/IGF-1 action in experimental animals after puberty should be undertaken, and additional simpler models should be developed to overcome these limitations.

2. The major pathological finding in long-lived GH-deficient and GH-resistant mice is decreased tumor burden. In humans, high plasma IGF-1 levels have been shown to be a risk factor for a variety of cancers. Conversely, high plasma IGF-1 levels have been shown to be protective against several age-related diseases such as osteoporosis, impaired glucose tolerance, type 2 diabetes mellitus, and cognitive decline. Thus, determining whether the effects seen in mouse mutants are not only cancer specific is important for translating these findings to humans.

3. The example of protective effects of reduced plasma IGF-1 levels that may be relevant to the real anti-aging effects in these models is provided by cellular resistance. However, the cellular work has been limited in several ways. First, it has been done almost exclusively in fibroblasts. Second, it has been done in serum-free media, which is expected to affect any cellular function, and there is no information about whether incubating these cells with IGF-1 would have deleterious effects on their response to stress. Investigating the role of IGF-1 in stress resistance and the universality of this interesting observation is still in the initial stages.

4. As noted for CR, a systems biology approach, developing new models, and more thorough testing for health span are important for the advancement of this line of research.

Conclusions

This article is a result of "brain storming" at the Summit meeting and emphasizes respective areas of consensus concerning priorities for research. It does not attempt to comprehensively cover the field (eg, other hormones such as insulin are extremely important in aging but were not part of this discussion). It also does not attempt to offer an in-depth scientific analysis or a complete list of references to all that was covered. The main objective is to simply identify directions and opportunities that are emerging, coupled to technological advances, development of additional models, and incorporation of a systems biology approach. Some of the necessary developments are in areas that are primarily descriptive but nevertheless necessary. Clear examples are the development of tools for assessment of healthy aging and development of new models. Thus, collaboration between the scientists and the funding agencies is needed to continue to provide breakthroughs and to lead to translational research.

Our optimism concerning likely progress on basic research and its practical applicability is based on past successes. It is fortified by the striking discovery by gerontologists of the sirt1 nutrient sensor, leading to the identification of resveratrol, which is already in a clinical trial to treat type 2 diabetes mellitus and may emerge as a treatment for other age-related diseases. Such an example sets a laudable goal for our research projects.

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