

Caloric restriction and its mimetics

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Caloric restriction is the most reliable intervention to prevent age-related disorders and extend lifespan. The reduction of calories by 10-30% compared to an *ad libitum* diet is known to extend the longevity of various species from yeast to rodents. The underlying mechanisms by which the benefits of caloric restriction occur have not yet been clearly defined. However, many studies are being conducted in an attempt to elucidate these mechanisms, and there are indications that the benefits of caloric restriction are related to alteration of the metabolic rate and the accumulation of reactive oxygen species. During molecular signaling, insulin/insulin-like growth factor signaling, target of rapamycin pathway, adenosine monophosphate activated protein kinase signaling, and Sirtuin are focused as underlying pathways that mediate the benefits of caloric restriction. Here, we will review the current status of caloric restriction. [BMB Reports 2013; 46(4): 181-187]

INTRODUCTION

Everyone desires a long and healthy life, and many researchers have investigated methods to overcome and to retard the aging process. The most well defined intervention of retarding aging is caloric restriction. Caloric restriction, also known as dietary restriction, is the reduction of food intake without malnutrition. Experimentally, caloric restriction means a reduction in calorie intake by 10-30% when compared to an *ad libitum* diet (1). Lifespan extension in response to caloric restriction is thought to be caused by a decreased rate of increase in age-specific mortality (2). It is widely believed that caloric restriction delays the onset of age-related decline in many species (1), as well as the incidence of age-related diseases such as cancer, diabetes, atherosclerosis, cardiovascular disease, and neurodegenerative diseases. Caloric restriction affects the behavior, animal physiology, and metabolic activities such as modulation of hyperglycemia and hyperinsulinemia, as well as

increases insulin sensitivity (3).

Reductions of protein source in the diet without any changes in calorie level have been shown to have similar effects as caloric restriction (4). Furthermore, restriction of individual amino acids has been shown to induce lifespan extension in some species, especially methionine restriction (5). Moreover, the restriction of tryptophan is believed to have a positive effect on longevity (6). Thus, several researchers have stated that this phenomenon occurs as a result of dietary restriction, not caloric restriction. However, other studies have indicated that protein and/or methionine restriction is not involved in the caloric restriction-induced lifespan extension (2).

HISTORY OF CALORIC RESTRICTION STUDIES

The first experimental evidence of the effects of food restriction on lifespan was provided by Osborne *et al.* (7). In the early 1900s, they reported that the restriction of food intake of rats retarded their growth, but prolonged their lifespan. However, their study did not get much attention since Robertson and Ray reported the results to show the correlation of longevity to growth rate of mice after three years (8). The most noted study of the effects of caloric restriction was conducted by 1935 by McCay *et al.* (9), who showed that restriction of food intake by 40% from the age of weaning extended lifespan of rat by up to two times. Their findings were confirmed by a series of experiments conducted by Walford and Weindruch using mice in 1986 (10). Weindruch reported that mice brought up under caloric restriction lived longer than a control group fed *ad libitum*, and that they also showed improved external appearance and physical conditions, as well as retardation of the onset of age-related diseases (10). To date, the effects of caloric restriction on lifespan and health had been demonstrated in many model animals from yeast to mammals.

MODEL ANIMALS FOR CALORIC RESTRICTION: FROM YEAST TO PRIMATES

The effects of caloric restriction on longevity and health have been reproducibly investigated in a wide range of laboratory animals, yeast, worms, fruit flies, and rodents, as well as some wild animals including cows and dogs (1). In addition, investigations of caloric restriction on non-human primates has been conducted in the last few decades, as have studies of caloric restriction on humans based on epidemiological data

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and volunteer studies.

Yeast

Saccharomyces cerevisiae, a single-celled budding yeast, is an excellent experimental model for the discovery of fundamental mechanisms associated with aging and genetic screening for mechanisms of longevity effects of caloric restriction (11). Caloric restriction of *S. cerevisiae* is performed via the reduction of glucose in growth medium. The limitation of glucose availability via growth in low-dose glucose medium (0.5% glucose) has been shown to extend the replicative lifespan of yeast (12). In addition, yeast with a mutation of the *Hxt1* gene, which is a sensor for glucose, lived longer than controls, further indicating that glucose limitation extends yeast lifespan (12). However, despite its usefulness, the yeast model system has a great weakness in that it is a unicellular organism that is very dissimilar to humans.

Nematodes

Nematodes (*Caenorhabditis elegans*) are the simplest experimental model animal among multicellular organisms, allowing investigations of intercellular and tissue changes in response to caloric restriction. Studies have shown that the reduction of their food source (*E. coli*) can extend the lifespan of nematodes (13). Dissimilar to the mammalian model system, the effects of caloric restriction on the lifespan of worms increases as the restriction increases to starvation. In the absence of bacteria, the lifespan of worms was reproducibly increased by up to 150% (14). The medium utilized for the *C. elegans* model system is well defined, allowing investigation of the roles of individual nutrients on caloric restriction benefits. In addition, the *C. elegans* model system is useful for identification of mechanisms of caloric restriction since it has a relatively shorter lifespan than other multicellular model systems as well as a great deal of known mutants related to lifespan.

Fruit flies

One of the most fascinating model systems for investigation of gerontology is the fruit fly, *Drosophila melanogaster*, which has a high similarity to human disease-related genes, as well as a relatively short lifespan (approximately 60-80 days). The relationship of the response to the dose of caloric restriction has been well established in *D. melanogaster*. In studies of *D. melanogaster*, the restriction of food is generally performed via the dilution of nutrients (especially yeast as a protein source). It seems that diet quality, not calorie per se, is important regulator of lifespan after caloric restriction at least in *Drosophila* since yeast restriction and carbohydrate restriction had differing effect on lifespan (15). Recent studies have shown that the balance of protein to non-protein energy ingested is the key determinant of lifespan in *Drosophila* (16).

Rodents

The rodent model system was the first system investigated for

benefits of caloric restriction on lifespan (17). Rodent models including rats and mice have a high similarity to human diseases and metabolism, making them the most practical. Caloric restriction interventions of rodents were first performed via the reduction of food supplemented daily, but the alternative protocol of alternate-day fasting or intermittent fasting was later developed (18). The alternate-day fasting intervention was confirmed to have beneficial effects such reduced insulin sensitivity, diabetes, and body weight, as well as extended lifespan (19).

Non-human primates

The beneficial effects of caloric restriction on non-human primates, especially rhesus monkeys (*Macaca Mulatta*), have been investigated by three independent groups, the National Institute of Aging (NIA), the Wisconsin National Primate Research Center (WNPRC), and the University of Maryland. At the University of Maryland, Hansen focused on obesity and diabetes in rhesus monkeys using short-term caloric restriction. She found that caloric restriction had a beneficial effect on body weight, insulin sensitivity, and diabetes (20, 21). The effects of caloric restriction on non-human primates longevity was investigated in two independent 20-year longitudinal adult-onset studies in rhesus monkeys (22, 23). In 2009, WNPRC reported their long-term study begun in 1989, which showed that caloric restriction extended the median lifespan of rhesus monkeys and reduced the onset of age-related diseases (22). Although their study was not completely finished, this report suggested that the benefits of caloric restriction on longevity are evolutionally conserved and controlled by conserved mechanisms. However, in 2012, NIA published another study about the effects of caloric restriction on rhesus monkey lifespan (23). Their study, which began in 1987, showed results that were somewhat different from those reported by the WNPRC. Although it was also ongoing study that showed beneficial effects of caloric restriction on age-related diseases, the young onset caloric restriction monkeys did not show advantages associated with age-related mortalities when compared to *ad libitum* monkeys. These opposing results seem to be caused by differences in the method of diet supplementation, the amount of calories provided to control animals, and/or the genetic background of the experimental animals. Nevertheless, the positive results from studies of non-human primates suggest that restriction of food intake can help humans have longer and healthier lives.

Humans

Owing to ethical and experimental limitations, investigations of human caloric restriction have not been actively conducted. As a result, studies of the benefits of caloric restriction on humans have primarily been restricted to epidemiological studies. However, many people follow food restrictions for religious or regional reasons. Studies of the effects of observance of Ramadan, during which time Muslims do not intake any

food or water between sunrise and sunset, showed conflicting results (24, 25). Another representative experimental study of human caloric restriction is the CALERIE (Comprehensive Assessment of Long Term Effects of Reducing Caloric Intake) program, in which healthy volunteers underwent the caloric restriction interventions for 2 years (26). The CALERIE groups have published several reports showing the benefits of caloric restriction on anti-aging, including increased insulin sensitivity, improvement of plasma lipid composition, and attenuation of oxidative stress (27, 28). However, caloric restriction does not seem to have a beneficial effect on age-related bone and muscle deterioration in humans (29, 30).

MECHANISMS OF CALORIC RESTRICTION

Hypotheses of anti-aging effect by caloric restriction

The underlying mechanisms of the effects of caloric restriction on longevity have not yet been definitely demonstrated. Indeed, there are hundreds of hypotheses that still must be tested before the mechanism responsible for extended lifespan in response to reduced food intake is fully understood. In early caloric restriction studies, there were some hypotheses; McCay *et al.*, proposed developmental delay hypothesis, the retardation of growth by caloric restriction increases lifespan (17). Pearl proposed the reduced metabolic rate hypothesis suggesting that caloric restriction reduces metabolic rates, followed by lifespan extension (31). In addition, the laboratory gluttons hypothesis tested whether ordinary overeating of laboratory animals compared to their wild compartment negatively affected their health, while the caloric restriction effects of laboratory animals were merely the rescue effect of excessive eating (32). However, these early hypotheses were almost all discarded owing to the conflicting results of various investigations.

The most reliable hypothesis of the anti-aging effect of caloric restriction is associated with the reduction of oxidative stress. Oxygen that enters the body is changed into reactive oxygen species (ROS) through cellular respiration, which subsequently attacks the macromolecules in the cells, resulting in the onset of age-associated changes. Caloric restriction is thought to reduce ROS production, which delays the aging processes (33). Numerous studies have supported this hypothesis. However, there is also considerable controversy associated with hypothesis. For example, the null mice of the antioxidant superoxide dismutase did not show accelerated aging, despite the presence of increased oxidative damage (34). In addition, the reduction of ROS production did not extend the lifespan of *Drosophila* (35). Thus, more information regarding the relationship between ROS, lifespan, and caloric restriction is needed.

Caloric restriction also reduces body temperature, and this decreased body temperature has been shown to be one of the mechanisms contributing to lifespan extension by caloric restriction. Specifically, the effects of caloric restriction were lost when animals fed a restricted diet were kept at higher tem-

perature (36). Overall, the data generated to date indicate that a complex combination of a variety of mechanisms may be involved in the beneficial effects of caloric restriction.

Molecular signals associated with caloric restriction

Although the mechanisms of caloric restriction have not been clearly defined, most gerontologists believe that the effects of caloric restriction on longevity is associated with nutrient-triggered signaling cascades, such as insulin/insulin-like growth factor-1 (IGF) signaling, and the target of rapamycin (TOR) pathway. Lifespan extension and retardation of aging processes are observed when the activity of these nutrient-triggered signaling pathways is reduced by mutations or chemical inhibitors.

Insulin/IGF signaling

Over intake of glucose, a major energy source in a variety of model animals, induces age-related diseases such as diabetes and cardiovascular disease. Increased glucose levels in serum followed by food intake promote the secretion of insulin hormone, which in turn activates insulin/IGF signaling. After binding with insulin, insulin receptor activates downstream factors such as PI3K/Akt/Ras and/or represses forkhead box O (FOXO) transcription factor, which is well-known to regulate stress response genes (37). Modulation of organismal longevity by insulin/IGF signaling has been well defined in various animals from yeast to mammals (38). Under caloric restriction conditions, systemic levels of insulin/IGF signaling are definitely decreased in many species. Thus, insulin/IGF signaling has been proposed as a mediator of longevity benefit by caloric restriction. However, it is still not clear whether the longevity extension effect of caloric restriction is dependent on the insulin/IGF signaling pathway. Flies containing the FOXO mutation still responded to caloric restriction (39), and the lifespan of long lived-Ames dwarf mice generated by mutation of growth hormone was also extended by caloric restriction (40).

TOR pathway

The other nutrient sensor signaling pathway known to regulate longevity is the TOR pathway, which is a well-known amino acid sensor that is evolutionally conserved from yeast to mammals. Activation of TOR by amino acids promotes protein synthesis via the activation of S6K and/or the inhibition of 4EBP, and the inactivation of TOR promotes degradation of damaged proteins and intracellular organelles via autophagy. Thus, decreased expression or activity of the TOR signaling pathway is known to extend lifespan in nematodes, flies, and rodents (41, 42). Since it plays a role as an amino acid sensor, the TOR pathway has been proposed as a mediator of caloric restriction. However, there are conflicting reports of the effects of caloric restriction on TOR expression in rodents (43, 44). Thus, further intensive investigations are required to enable a precise understanding of the mediators of caloric restriction.

Adenosine Monophosphate -activated Protein Kinase (AMPK) signaling

AMPK is emerging as a key nutrient-triggered signaling pathway underlying the lifespan extension effect of caloric restriction. Under energy deprivation conditions, LKB phosphorylates and activates AMPK, which in turn stimulates the processes to generate ATP. The nematode model system has generated evidence in support of the function of AMPK on lifespan extension by caloric restriction. Worms overexpressing AMPK (*aak-2*) lived longer than controls, and glucose restriction increased *aak-2* activity (45). Furthermore, it was reported that the lifespan extension effect by caloric restriction was dependent on *aak-2* in a *C. elegans* model (46). In the *Drosophila* model system, activation of AMPK activity via the overexpression of LKB1 extended the lifespan (47). In addition, a recent study showed that the tissue specific overexpression of AMPK in muscle and abdominal fat body extended the fly lifespan, and that supplementation of adenosine could modulate the beneficial effects of caloric restriction, which are associated with the activation of AMPK (48). However, it is still not clear whether AMPK is a mediator of the effects of caloric restriction on longevity in mammalian systems (49).

Sirtuin

At the beginning of the 21st century, a new factor, Sirtuin, gained a great deal of attention as a mediator of caloric restriction. Sirtuins are evolutionally conserved nicotinic amide (NAD)-dependent histone deacetylases. In calorie restricted environments, the expression and activity of Sirtuin is increased in many tissues, including adipose and brain tissue (50). The overexpression of Sirtuin in worms and flies increased lifespan (51, 52), and mutants of Sirtuin do not show lifespan extension by caloric restriction (12). The roles of Sirtuin as a mediator of caloric restriction have also been shown in the mammalian model animal. Transgenic mice expressing SIRT1, which is the most thoroughly investigated mammalian Sirtuin, showed similar phenotypes to that of food restricted mice (53). In addition, the extension of longevity in response to caloric restriction was not observed in mice from which SIRT1 had been deleted (54). However, there is conflicting evidence regarding the role of Sirtuin as a caloric restriction mediator. Indeed, some studies showed that various caloric restriction conditions did not activate Sirtuins, and the phenotype of Sirtuin overexpression did not exactly coincide with that of caloric restriction (55).

Caloric restriction results in systemic and global changes in the body, and the above signaling pathways are tightly cross-linked. Therefore, it is not likely that the global changes caused by caloric restriction are the result of a single genetic factor. Accordingly, more comprehensive and macroscopic analyses will be required to interpret and comprehend the mechanism of caloric restriction.

CALORIC RESTRICTION MIMETICS

Although the beneficial effects of caloric restriction on lifespan and health have been clearly demonstrated, it is difficult to implement such restrictions in our lives. To overcome these difficulties, gerontologists and biologists are attempting to develop drugs to mimic the beneficial effects of caloric restriction without the need for diet limitations. Such medicines are known as caloric restriction mimetics (CRM).

Resveratrol

Resveratrol is a polyphenol compound isolated from the skins of red grapes. Resveratrol is currently the most thoroughly studied CRM. As a CRM, resveratrol was first identified by Sinclair through screening of small molecular libraries for compounds that activate Sirtuin and extend lifespan in a yeast model (56). In that study, they demonstrated that resveratrol can mimic the benefits associated with caloric restriction, and that caloric restriction did not further extend the lifespan of yeast grown in resveratrol supplemented medium. Resveratrol was subsequently shown to extend longevity in worms, flies, fish, and obese mice (57, 58). However, a recent study showed that resveratrol had no effect on the longevity of mice fed a normal diet (59). Although the longevity extension effect of resveratrol is not yet certain, it is accepted that resveratrol can improve health and prevent age-related diseases. Further investigations and clarifications are required to verify whether resveratrol is a true CRM.

Rapamycin

Rapamycin, an antibiotic, immune-suppressor drug, is another proposed CRM that has shown a longevity benefit. After studies showing the extension of replicative lifespan of yeast via inhibition of the TOR signaling in response to rapamycin treatment (60), it was reported that rapamycin extends the median and maximum lifespan of 20-month-old mice accompanied with a decrease in TOR activity (61). Since then, many studies have been conducted to ascertain the function of rapamycin as a CRM. However, it is important to note that there is evidence showing adverse side-effects of rapamycin such as an increase in the incidence of diabetes (62).

Metformine

Metformin is another CRM of interest to gerontologists. Metformin is a biguanide used as a drug for treatment of type-2 diabetes that increases insulin sensitivity and activates AMPK. Metformin received a great deal of attention after it was identified in a screening assay of drugs showing similar transcriptional profiles to that of caloric restriction in mice (63). Moreover, metformin was shown to have a caloric restriction-related longevity benefit mediated by the activation of AMPK in *C. elegans* (64). Metformin also has a beneficial effect on other aspects of the aging process such as a decrease in age-related disease incidence. However, the longevity benefit

of metformin was not observed in a *Drosophila* model in a recent study (65), or in the non-disease rodent model (66). The appearance of evidence showing that metformin is ineffective on longevity in some species has led to uncertainty as to whether metformin is a true CRM.

CONCLUSIONS

As the number of elderly people who cannot undergo physical activities has increased, anti-aging has become one of the ultimate goals of gerontologists. Although it has been 75 years since the beneficial effects of caloric restriction on animal health were first reported, the underlying mechanism of longevity extension in response to food restriction has still not been identified. Further organized and global investigations encompassing various research fields using well-developed genetic model animals and well-controlled practical human studies will enable the aging process to be controlled, thus allowing humans to live healthier and happier lives in the near future.

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