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Aging: Is caloric restriction anti-aging?

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Γήρανση: Είναι ο θερμιδικός περιορισμός αντι-γηραντικός;

Περίληψη στο τέλος του άρθρου

The mechanisms that lead to the progressive aging process of an organism are multiple and complex. The theories of aging are categorized into damage-based, which focus on the progressive accumulation of damage throughout life, and program-based, which are based on the concept that aging is the outcome of a genetic program. The multiplicity of theories on the mechanisms of aging makes the search for ways to prolong life more difficult. Searching for a way to increase the life span and at the same time delay the onset of age-related diseases has led to recommendation of the most widely used method, which is called caloric restriction. Caloric restriction is based on the reduction of food intake while, very importantly, maintaining appropriate nutritional intake. Caloric restriction gives promising results in a vast range of animal models, such as prolonging their life span and delaying the onset of age-associated diseases, but the relevant results from research on rhesus monkeys are still pending. Questions are raised, however, regarding the efficacy of its application in humans. The main reasons are that the nutrient composition of a calorie-restricted diet is very important, and that maintaining such a diet throughout life will be quite testing and largely prone to failure, taking into account its still questionable benefits. For this reason, the field of "caloric restriction mimetics" is gaining ground and stimulating interest where humans are concerned. This area focuses on identifying compounds that, when consumed, would confer similar life prolonging and age-delaying phenotypes as caloric restriction, but with less effort. Caloric restriction mimetics sounds promising but research in the field is still in the early stages.

Key words

Aging
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Longevity

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1. INTRODUCTION

Aging of organisms is not the outcome of wear and tear alone, but is also influenced by genetic factors, which result in failure to repair the wear and tear, such as at the termination of the reproductive phase in females by the onset of menopause due to absence of oogenesis.[1] The causes of aging have been the subject of debate for a long time. Some researchers suggest that aging is the outcome of the incapacity of the organism to repair itself,[2,3] while others support the idea that aging is the outcome of a well-programmed procedure that occurs as part of the developmental cycle.[4] While the latter theory applies for certain species, the human aging process is much more gradual. It is far more likely that human aging is the result of a "molecular clock" that regulates the process of aging, but that runs at different rates, influenced by external factors. There are several genes in mice that can increase the maximum life span by even more than 50% while delaying the appearance of age-related diseases, proving that genetics regulate aging to a certain degree.[5] Further evidence was offered by the accelerated pace of aging observed in organisms that carry certain mutations, such as in the case of Werner's syndrome.[6] In 1935, it was discovered that limiting calorie intake in rats delayed the onset of aging.[7] This process, known as caloric restriction, was further studied in mice where it lead to a 50% increase in life span, delay in the onset of age-related diseases and their development, and decrease in the rate of aging.[8]

2. THEORIES OF AGING

The processes and mechanisms that lead to aging are many, as are the theories on how aging progresses. The theories of aging can be divided into two categories: those that are damage-based, otherwise known as stochastic, i.e., that damage is accumulated throughout the entire life span,[9] and those that are program-based, i.e., that the onset of aging is genetically regulated.[10] Just as for many of life's stages, it is wise to recognize that aging is the result of many different processes. Damage and genetic factors

both contribute to aging, and influence the timing of its onset to some degree.

It is difficult to study the biology of aging in humans, due to their extensive life span. It is therefore important to use appropriate models, some of which are human cells, mice, rats, yeast and Drosophila. No model is considered perfect but it is safe to assume that animals evolutionary closer to humans are more appropriate for human biology extrapolations rather than distant species. There is a great diversity of aging phenotypes in nature, but mammalian aging has a similar phenotype in most species[11,12] and includes reproductive decline, osteoporosis and arthritis.

The rate of aging of a certain species is estimated using the maximum life span (T_{max}) of this species, which, although not perfect, is the best way available. In general, in animal species body mass correlates with maximum life span,[13,14] despite the fact there are some exceptions to this, such as bats and birds, that live much longer than expected based on their size. The most obvious explanation for this is that smaller animals are more prone to predators and therefore have a shorter life span, which in turn influences their aging process. Bats and birds, being able to fly can evade predators more easily, increasing their maximum life span. When looking to find a correlation of a certain factor with the maximum life span between species, it is important, therefore, to take into consideration body mass differences.[15]

2.1. Damage theories

2.1.1. Rate of living theory. The "rate of living theory" relates the maximum life span to the metabolic rate, suggesting that animals with a lower metabolic rate live longer.[16] One hypothesis was that caloric restriction reduces the metabolic rate, thereby increasing longevity, although some studies showed no effect of caloric restriction on the metabolic rate.[17] The way the metabolic rate was estimated (oxygen consumption at rest) did not correct for the species body mass (i.e., large or small animal), which thereby resulted in incorrect calculation of the species metabolic rate. Subsequent research showed that mutations in the tau protein lead to increased life span.[18] More recent data showing that correctly calculated metatolic rate does not correlate with maximum longevity[19] sharply reduce the credibility of the "rate of living" theory.

2.1.2. Free radical theory. Another theory of aging is that of the effects of free radicals and oxidants, otherwise known are reactive oxygen species (ROS). ROS can damage cellular components,[20] and since they accumulate with age, it was argued that aging results from the damage caused by ROS.[21] The organism protects itself against ROS by using antioxidants, such as vitamins C and E, and enzymes, including superoxide dismutase (SOD), catalase and glutathione peroxidase. Overexpression of SOD1 and catalase in short-lived strains of Drosophila was shown to lead to increased longevity, but not delayed onset of aging.[22] Longer-lived strains of Drosophila were not examined in these experiments, but it is known that they have increased levels of antioxidant enzymes.[23] In mice, however, overexpression of SOD1 did not lead to prolonged life span[24] and mice heterozygous for a SOD2 knock-out accumulated greater oxidative damage but did not suffer effects on longevity or the aging process.[25]

Two enzymes that catalyze the repair response due to ROS generated damage are methionine sulfoxide reductase A (MSRA) and 8-oxo-dGTPase. While MSRA has been linked to increased longevity in fruit flies that overexpress this enzyme[26] and reduced longevity in those that lack it,[27] 8-oxo-dGTPase caused no change in mouse longevity apart from increasing cancer incidence.[28] Overexpression or knocking-out of antioxidants in mice led to disappointing results.[29,30] Feeding mice with antioxidants in one study decreased oxidative damage and prolonged life, but did not delay aging,[31] while other studies question the ability of antioxidants to achieve any prolongation of life at all.[32] These data suggest that mammals are already in an optimized state as far as antioxidants are concerned, and that while they may help the organism to stay healthy, they do not influence its aging process.

ROS is produced in the mitochondria, the location of the cellular metabolism. Several pathological conditions linked to the mitochondria that involve increased leakage of ROS[33,34] do not result in accelerated aging, but often lead to central nervous system (CNS) disorders.[35] Deficiencies in complex I, cytochrome C and vitamin E have been associated with neurodegenerative disorders.[34,36,37] It is possible either that ROS affect post-mitotic cells such as neurons, or that mitochondrial diseases mainly target the CNS.[38]

2.1.3. DNA damage theory. The original hypothesis was that accumulation of damage to the DNA leads to aging.[39] Later, when mutations in the DNA were discovered, this theory evolved to include the hypothesis that accumulation of mutations may also lead to aging.[40] Several progeroid syndromes link aging to DNA repair/metabolism.[41] Despite this, knock-out of the DNA repair protein Pms2 in mice failed to accelerate the onset of aging.[42] In addition, mutations of the p53 DNA repair protein fail to accelerate aging, despite affecting longevity and increasing cancer incidence in humans.[43]

While DNA mutations and chromosomal abnormalities increase with age in mice[44–46] and humans[47,48] distinction cannot be made between cause and effect in the aging process. In order for the DNA damage theory to be correct, it would be expected that an increase in DNA repair mechanism would prolong life. However, studies in mice showed that overexpression of the *MGMT* DNA repair gene did not achieve an extended life span or delay aging onset.[49]

Free radicals damage the DNA, but given that so many changes to the nuclear DNA do not affect aging, ROS damage caused to mitochondrial DNA (mtDNA) could be considered as a more relevant cause of aging. While the relationship of mtDNA oxidative damage to aging is questioned,[50] recent data show that disruption of the mitochondrial DNA polymerase leads to an increased aging phenotype,[51] due, however, not to increased oxidative stress but rather to increased apoptosis and accumulated mtDNA damage.[52,53] To summarize, DNA changes influence aging over time but it remains unknown which mechanism is followed. Mitochondrial DNA, rather than nuclear DNA, is linked to age-related diseases and aging.[54]

2.2. Programmed theories

2.2.1. Endocrine theory. The levels of certain hormones, such as the growth hormone (GH), decrease with age.[55] In rodents, caloric restriction has been associated with hormonal changes, such as drop in plasma insulin[56] and IGF-1 levels.[57] Brain-specific overexpression of a gene that reduces appetite (urokinase-type plasminogen acticator) results in a 20% decrease in food consumption and body mass and a 20% increase in longevity.[58] Another example is seen in mice homozygous for *Pit1,* which have lower growth hormone (GH) and IGF-1 levels, and show an increase in maximum life span and a delay in aging onset.[59] Mice mutated for *Prop1*, which encodes a transcription factor that regulates *Pit1*, also live longer.[60] When *Prop1* mutated mice were kept on a calorie restricted diet, their longevity was further increased, suggesting the action of two distinct mechanisms that extend life span.[61] Humans carrying mutations on *Prop1* live slightly longer.[62] These findings support a link between the neuroendocrine system and aging.

2.2.2. Developmental theory. The *dauer* pathway in *C. elegans*, which can be activated by starvation, thereby mimicking caloric restriction, results in significant life extension[63] by arresting development,[64] suggesting that development and aging are linked. In higher animals, there is a correlation between the time of sexual maturity and life span,[65] so that delay in sexual maturation would lead to a prolonged life span. This suggests either that aging and development are regulated by the same genetic mechanisms, or that reaching a landmark time point in development triggers the onset of aging.

3. CALORIC RESTRICTION

In order to achieve a true anti-aging effect, a specific method needs not only to lead to an increased life span but also to delay the onset of aging and its effects, such as age-related diseases. Caloric restriction, otherwise known as diet restriction, is the best way to date of delaying the onset of aging, which it effects by reducing the intake of calories while maintaining appropriate levels of essential nutrients such as minerals and vitamins.

The way in which caloric restriction works is still not understood, despite this method having been used for over 70 years.[6] The favorable impact of caloric restriction on blood pressure, obesity, diabetes mellitus, autoimmune diseases and cardiovascular diseases presents very important implications for human health and has generated extensive research. One suggestion is that caloric restriction forces the organism to make better use of its energy, thereby optimizing its metabolism. Since caloric restriction can influence development, as mentioned earlier, it could be argued that it modifies expression of the genetic mechanism of the organism, which subsequently affects aging.[66] Other suggestions are that caloric restriction prolongs life and delays the onset of age-related disease by postponing immunologic aging,[67] decreasing oxidative damage[68] and preserving protein synthesis in old age.[69]

A number of nutrient sensitive proteins have been implicated in longevity, including the sirtuins, forkhead transcription factors and the metabolic regulator mTOR; these proteins therefore may also be important in caloric restriction. Gene expression profiling has allowed comparison of transcription profiles in post-mitotic tissues of control and diet-restricted mice. This comparison revealed that caloric restriction prevented age-related changes, but also transcriptional changes, which are associated with the process of caloric restriction and are not age-dependent.[70] In the heart, transcriptional changes included increased turnover of structural proteins and a shift from fatty acid to glucose metabolism.[70] One of the factors that could be responsible for the metabolic reprogramming induced by caloric restriction is the transcriptional co-factor PGC-1a, the mRNA levels of which increase under caloric restriction.[71] In addition, PGC-1a influences the balance between car-

bohydrate and fat metabolism by co-activating a family of transcription factors.[72] Recently, mice under oxidative stress and mice on caloric restriction were shown to use PGC-1a alterations in stability, localization and activity, which are processes that are regulated by SIRT1 and GSK3β, in their response to caloric restriction and stress.[73]

3.1. Caloric restriction in animals

Several types of calorie-restricted diets are used in animal research, including protein restriction, diet restriction, intermittent fasting and food restriction. Diet restriction involves a group of *ad libitum* fed animals (controls) whose daily consumption is monitored and a group of diet restricted mice, which typically receive a certain percentage (60% or 70%) of the control group's daily intake of the previous day. The two groups are age-, sex- and strain-matched. When the first signs of advanced age occur, the diet restricted animals' food intake is no longer coupled to that of the control group, but is kept constant, making their diet less restricted with age. Another, commonly used method is to feed the diet-restricted mice a pre-determined percentage (60% for example) of the age-, sex- and strain-matched control adult animals' average daily food intake, and to maintain this for their entire life. When this method is used, the control animals are often not allowed *ad libitum* access to food but instead are given the average daily food intake value that was used to calculate the diet-restricted group's portions.[74] While some groups[75] give food fortified in vitamins and minerals, it is probably not important to do so due to the purified laboratory mouse diet. The calorie restricted mice are ultimately smaller and weigh less, making the amount of food they consume greater per body weight unit compared to that of the larger and heavier control group mice.

Another method of calorie restriction is intermittent dieting. This method is applied by instituting 24hr gaps in feeding. Despite the concerns about stress during the 24hr period with no food, this diet results in protection against age-related diseased and is associated with an increased life span.[76] Caloric restriction in rhesus monkeys follows a specific meal schedule rather than permitting *ad libitum* access to food.[77]

In various studies on Prop1df (Ames dwarf) and Ghr/bp knock-out (Laron dwarf, GHRKO) mice, longevity was compared between mice on different caloric restricted diets (standard diet, casein diet, soy-based diet and high or low isoflavone diet). The longevity of all mutant mice was increased on all the diets, but the variation was striking, ranging from 35% to 51% in Amed dwarf mice and from 23% to 51% in GHRKO mice.[60,78,79] This shows that differences in diet composition can have a significantly different effects on the outcome, and that it is therefore important to examine which protocol of caloric restriction was used in each work.

Mice on a calorie-restricted diet have been observed to have a 40% greater life span,[7] a delayed aging phenotype and a reduction in age-related diseases, although not all mouse strains show such results. Wild-derived mice do not show extended life span despite indications of a level of protection against cancer,[80] which puts into question the data from laboratory kept mice, but the discrepancy may possibly be the result of unestablished caloric restriction conditions for the wild-derived mice. In rats, caloric restriction delays the rate at which mortality increases with age.[30] Mice on diet restriction were also able to respond better to exogenous stimuli and had reduced levels of autoimmunity, therefore staying "younger" for longer.[81]

Research in non-human primates is still under way, but is incomplete due to their long life span. Although conclusions from primate research are not yet available, some preliminary data have been published. Rhesus monkeys show improved insulin sensitivity, decreased blood glucose and insulin levels, and lowered body temperature, just as mice, when their diet is restricted.[82,83] Rhesus and squirrel monkeys show decrease in neoplastic events in calorie-restricted subjects.[84]

3.2. Caloric restriction in humans

Caloric restriction in humans might be beneficial, but it is important to realize that *ad libitum* eating can also be considered as overeating, with caloric restriction being the return to a normal eating level. Research on the effects of caloric restriction in humans is exceptionally difficult due to the expensive and time-consuming nature of such studies. In addition, most of the world's calorie restricted individuals are also malnourished. However, some studies on closely monitored calorie-restricted individuals over a short period (6 months) documented a decrease in blood pressure, cholesterol and serum lipids, and improved vascular function.[85,86] Observation of individuals living in Okinawa where the diet is comparable to that of rodents on calorie restriction, showed an increased incidence of centenarians.[87] People forced to reduce their calorie intake for two years due to lower than expected food production, but avoiding malnutrition, showed several changes in their physiology similar to those seen in calorie-restricted

rodents. These include a decrease in weight, blood pressure, total serum cholesterol and triglycerides, fasting glucose and leukocyte count.[88]

4. CALORIC RESTRICTION MIMETICS

In spite of the encouraging data derived from animal studies on the benefits of caloric restriction, it is safe to assume that most humans would not be able to strictly follow such a specific diet, thereby rendering caloric restriction as an inefficient method of prolonging the human life span. This rationale led to the development of a new area of research called "caloric restriction mimetics", which focuses on finding a way in which compounds can mimic the effects of caloric restriction. It is important to note that research in caloric restriction mimetics is performed on certain animal models and further research is required before stable conclusions can be drawn.

One compound used to mimic caloric restriction is 2-deoxyglucose, but a disadvantage of this candidate is that it can be toxic. Research has established concentrations at which toxicity is avoided, while achieving the objective of studying the difference in effect of non-restricted dietary intake between the control group and the 2-deoxyglucose treated group. Rats at the highest concentrations of non-toxic 2-deoxyglucose showed a modest 5–10% increase in life span, a slight reduction in weight, and a consistent, but not statistically significant, drop in blood glucose levels.[89,90] These results are consistent with the effects calorie restriction would have induced.

The most well-known molecules used in caloric restriction mimetics are a group called sirtuin-activating compounds (STACs), such as resveratrol. Resveratrol, found in a high concentration in red wine, is known to have anti-oxidant properties and to reduce the incidence of cancer, while some of its other beneficial effects are cardiovascular disease prevention and life span extension.[91,92] Sirtuins are genes, which when activated, increase the life span by silencing other genes.[93] Research in yeast identified resveratrol as the main activator of the sir2 sirtuin.[94] Sir2 in mammals is SIRT1, which is activated by caloric restriction.[95] However, SIRT1 inhibition has been linked to neuron protection and could play an important role in neurodegenerative diseases such as Parkinson's disease.[96] While resveratrol has been shown to increase the life span of obese mice,[97] more recent research showed no effect on the maximum life span of mice, despite improvement in age-related changes.[98] More potent sirtuins than resveratrol are currently being developed and investigated, and show promising results in yeast[99] and obese mice.[100] Despite all this research, it is important to keep in mind that there is still no concrete evidence for a positive effect of caloric restriction mimetics in actually delaying the aging process.

ΠΕΡΙΛΗΨΗ

Γήρανση: Είναι ο θερμιδικός περιορισμός αντι-γηραντικός;

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Οι μηχανισμοί που οδηγούν έναν οργανισμό στην πρόοδο του γήρατος είναι πολλοί και περίπλοκοι. Οι θεωρίες του γήρατος κατηγοριοποιούνται σε θεωρίες φθοράς, που απασχολούνται περισσότερο με τη σταδιακή αύξηση της φθοράς του οργανισμού σε όλη τη διάρκεια της ζωής, και σε θεωρίες «προγραμματισμού», που θεωρούν ότι το γήρας είναι το αποτέλεσμα γενετικού προγραμματισμού. Οι ποικίλες θεωρίες για τους μηχανισμούς του γήρατος καθιστούν δύσκολη τη διαδικασία εύρεσης τρόπων αύξησης της διάρκειας ζωής. Η έρευνα για την επίτευξη μεθόδων μακροζωίας και παράλληλα επιβράδυνσης της εμφάνισης ηλικιακά σχετιζόμενων ασθενειών έχει οδηγήσει στην ευρέως χρησιμοποιούμενη μέθοδο του θερμιδικού περιορισμού. Ο θερμιδικός περιορισμός βασίζεται στον περιορισμό της καθημερινής τροφικής κατανάλωσης, ενώ διατηρεί την κατανάλωση κατάλληλης θρεπτικής ποιότητας. Ο θερμιδικός περιορισμός δείχνει υποσχόμενα αποτελέσματα σε μεγάλο αριθμό ζωικών μοντέλων που έχουν χρησιμοποιηθεί, όπως για παράδειγμα επιμηκύνοντας τη διάρκεια ζωής και καθυστερώντας τις ηλικιακά σχετιζόμενες ασθένειες. Παρόλα αυτά, τα αποτελέσματα από τις έρευνες σε πιθήκους αναμένονται ακόμη. Ωστόσο, υπάρχουν ερωτήσεις σχετικά με τη δραστικότητα αυτής της μεθόδου στους ανθρώπους. Οι λόγοι είναι ότι η σύνθεση των θρεπτικών ουσιών σε μια δίαιτα θερμιδικού περιορισμού είναι πολύ σημαντική και ότι η διατήρηση αυτής της δίαιτας κατά τη διάρκεια όλης της ζωής είναι αρκετά δύσκολη και με μεγάλες πιθανότητες αποτυχίας, χωρίς να ληφθούν καθόλου υπόψη τα ακόμη αμφισβη-

τούμενα οφέλη. Γι' αυτόν το λόγο, ένας νέος τομέας έρευνας έχει δημιουργηθεί, που ονομάζεται «μίμηση θερμιδικού περιορισμού» και ο οποίος κερδίζει έδαφος σε ενδιαφέρον όσον αφορά στους ανθρώπους. Ο συγκεκριμένος τομέας εστιάζει στον προσδιορισμό ουσιών που, όταν καταναλωθούν, θα προκαλέσουν παρόμοιους φαινότυπους μακροζωίας και αργοπορίας γήρατος, όπως ο θερμιδικός περιορισμός, χωρίς μεγάλη προσπάθεια. Η μίμηση θερμιδικού περιορισμού φαίνεται να είναι πολλά υποσχόμενη αλλά βρίσκεται ακόμη σε πρώιμα στάδια έρευνας.

Λέξεις ευρετηρίου: Γήρανση, Θερμιδικός περιορισμός, Μακροζωία

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