Aging, Calorie Restriction and Calorie Restriction Mimetics

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ABSTRACT:

In line with the increase in the number of older people in the world, the focus of scientists is directed at examining mechanisms of the aging process as well as establishing strategies/interventions in order to slow down aging and achieve longevity. On preclinical testing models, the most effective strategy for this purpose, as well as for the purpose of delaying age-related diseases, nutritional intervention- restriction of calorie (CR) has been demonstrated, but also some alternative forms of calorie restriction. Possible undesirable effects of restriction are still in the testing phase, and it is known that it is generally difficult to implement in humans. Therefore, the new area of research in gerontology has become the discovery and examination of the effects of compounds that mimic the effects of caloric restriction, so called caloric restriction mimetics (CRM). These compounds include numerous compounds of natural origin but also approved medicaments with certain indications. This overview summarizes the latest data on known mechanisms of caloric restriction and more familiar caloric restriction mimetics.

KEYWORDS: aging; caloric restriction; caloric restriction mimetics; sirtuins; resveratrol;

SAŽETAK:

STARENJE, KALORIJSKE RESTRIKCIJE I KALORIJSKO RESTRIKCIJSKA MIMETRIKA

U skladu s povećanjem broja starijih ljudi u svijetu, fokus znanstvenika usmjeren je na ispitivanje mehanizama procesa starenja kao i na uspostavljanje strategija / intervencija kako bi se usporilo starenje i postigla dugovječnost. Na predkliničkim modelima ispitivanja, najučinkovitija strategija za ovu svrhu, kao i za odgađanje bolesti povezanih s dobi, pokazana je prehrambena intervencija - ograničenje kalorija, ali i neki alternativni oblici ograničavanja kalorija. Mogući neželjeni učinci restrikcije još su u fazi ispitivanja, a poznato je da je to općenito teško provesti kod ljudi. Stoga je novo područje istraživanja u gerontologiji postalo otkriće i ispitivanje učinaka spojeva koji oponašaju učinke kalorijske restrikcije, takozvane mimetike kalorijske restrikcije. Ti spojevi uključuju brojne spojeve prirodnog podrijetla, ali i odobrene lijekove s određenim indikacijama. Ovaj pregled sažima najnovije podatke o poznatim mehanizmima kalorijskog ograničenja i poznatijim mimeticima za ograničenje kalorija.

KLJUČNE RIJEČI: starenje; kalorijska restrikcija; kalorijsko restrikcijska mimetrika; sirtuini; resveratrol;

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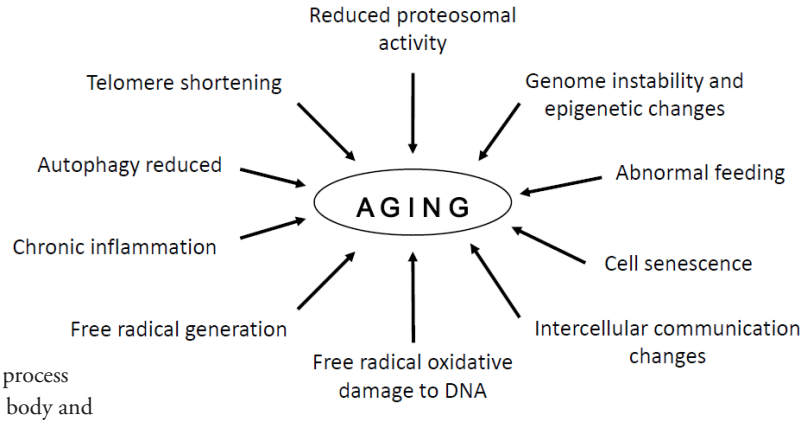

Figure 1. Some features that contribute to aging prosess and age-related diseases. Modified according to reference (2).

Introduction

Aging is an inexorable, complex and progressive but natural process that results in persistent worsening of the functioning of the body and increased morbidity and mortality. As a result of improved health care, the rate of the elderly population is steadily rising in most developed countries and is one of the biggest social challenges of today. Among other things, the process is associated with cellular senescence, shortening telomeres, genomic instability, epigenetic changes, intercellular communication changes, mitochondrial dysfunction, stem cell depletion, chronic inflammation, loss of protease and the like[1]. Figure 1 shows some of the factors contributing to aging progression and age-related diseases.

All these changes also increase the tendency of the elderly toward various pathological conditions, which are most commonly associated with persistent chronic inflammation[3]. Therefore, the primary focus of modern medicine is the treatment of specific age-related diseases. In the scientific community, more than 300 theory of aging processes are discussed, and the theory of oxidative stress seems to be the most comprehensive[4]. According to the Blagosklonny and Hall proposals, aging can be understood, for example, as "a type of unregulated continuation of normal developmental processes and related cellular programs with special emphasis on growth"[5], and this theory is complemented by inflammatory aging theory[6]. Inflammation occurring in older people is defined as subclinical, chronic and sterile one, with the accumulation of senescent cells, characterized by pro-inflammatory secretory phenotype[7].

However, prolonged life expectancy does not yet imply a healthy life span since aging is associated with a number of chronic diseases, such as diabetes, cancer, cardiovascular and neurodegenerative diseases[8]. Furthermore, the greater prevalence of age-related diseases is necessarily reflected in the cost of health care. Therefore, the scientific community around the world makes significant efforts to discover strategies for achieving so-called healthy, but also active aging. The term "Active Aging" includes the optimization of various opportunities for health, participation in life processes and the necessary security, all to improve the Quality of Life of older individuals[9]. Research in the field of aging is largely carried out on laboratory models and is directed towards various genetic and pharmacological interventions in the aging process itself[10]. Although they have been going on for decades, the translating of the obtained results into the human population is still questionable. Interventions for the purpose of healthy aging and longevity should include the attenuation or deceleration of some, if not all, already mentioned molecular-cellular changes, which include the aging process, the processes of inducing pathology associated with aging, and in particular, processes of deregulated sensing of nutrients or energy[1]. It has been shown that various interventions, including genetic manipulation, some drugs implementation and calorie limitation, extend the lifespan of several experimental models[11]. The superiority of some of these strategies in extending life expectancy has not been known until recently. However, the meta analysis of the published studies with Caenorhabditis and Drosophila, using the appropriate algorithm, emphasized the overall advantage of calorie (or energy) restriction (CR) in slowing down the aging process and prolonging the lifespan[12]. It is therefore considered that more acceptable, anti-aging strategies, are those strategies that include environmental factors, to reduce the number of risk factors of bad health[13]. Accordingly, one of the leading concepts of gerontology is a non-genetic, non-pharmacological CR strategy or a reduction in food intake, but without undernutrition, i.e. CR with optimal nutrition. The concept is not new, as through the history numerous communities promoted health benefits of dietary restrictions, including the ancient Greeks and Romans. Numerous studies, mainly on various model organisms, have shown that CR slows aging, prolongs the period of healthy life, postpones the onset of various age-related diseases and prolongs life expectancy[14,15]. CR as the idea of encouraging longevity originates from 1917 by Osborne et al. (16), who noticed after restriction of food in the rats, a reduced growth but extended life span. Later, this association was experimentally verified and confirmed by numerous research groups on animal models ranging from fungi to mammals[17,18]. Research results on non-human primates[19] are consistent with other models.

Because of the ethical limitations there are a small number of studies on the human sample (mostly epidemiological studies are conducted. Results of one of the few researches on the human sample, Comprehensive Assessment of Long Term Effects of Reducing Caloric Intake Energy Research (CALERIE)[20], confirmed the results of Biosphere 2 [21], i.e. a previously conducted two-year study of some anti-aging effects[22]. However, some undesirable effects of CR have been identified in this and other studies[23,24]. Given the possibility of the unwanted effects of long-lasting CR, questionable adhering to CR regime throughout life or for a long time, newer research as an alternative strategy involves the detection and development of CR mimetics (CRMs). This concept was first introduced by Lane et al. in 1998, proposing 2-deoxy-D-glucose (2DG) as CRM[25].

The data selected for this review were collected by searching the PubMed database, by the use of the following terms: lifespan, aging, healthspan, caloric restriction, dietary restriction, CR-mimetics. The focus of this paper is the possible CR mechanisms and the more familiar CRM. Additional searches were made in accordance with the names of specific pathways and pharmacological agents. Articles published in English between 2000 and 2019 were included. A limited number of selected articles and reviews is in accordance with the guidelines of the journal. We apologize to the authors of other excellent review articles that are not included in the text for this reason.

Calorie restriction

Calorie restriction (or energy restriction) means reduced caloric intake, less than 20% (mild CR) and 50% (severe CR) than the body needs[26] or reduced intake of food but without malnutrition. Nu-

merous studies have shown that CR induces pleiotropic, favorable changes in terms of longevity and extension of healthy life span, or postponement of the beginning of age-related diseases, regardless of whether CR is short or long-term practice. These include postponement of sarcopenia, brain atrophy, protection from arthritis and cardiovascular disease, prevention of age-related diabetes, reduction of incidence and progression of cancer, protection of colon health, maintenance of cognitive functions, etc.[27] Also, CR includes changes such as: modulation of important regulatory pathways through expression and stimulation the activity of key enzymes of metabolism, decreased oxidative damage of important biomolecules, increased antioxidative defense, modulation of mitochondrial activity, enhanced clearance of degraded biomolecules, reduction of chronic inflammation, control of cell repair mechanisms on molecular (eg. DNA repair, protein repair, membrane lipid repair), subcellular (autophagy), cellular and tissue levels (apoptosis) etc.[28]

Generally practiced CR methods include reduced consumption of food, change of nutritional ingredients, digestion inhibition and absorption of nutritional ingredients, inhibition of appetite and satiety and CRMs[28]. The implementation of the CR regime in the general human population is bad and may be unsafe for some persons, depending on their age or body weight. Long-lasting CR can, in fact, result in malnutrition, so the effects of CR variants, eg. Intermittent Fasting Diatery restriction (IF-DR), or occasionally starvation (every other day) are also tested. Due to the occasional nature of fasting, IF-DR approach seems to relieve the constant hunger effect, experienced by CR practitioners [29]. Some studies show that reduced intake of protein sources without calorie change[30] or even restriction of certain amino acids, such as methionine or tryptophan[31], has a similar effect as CR. This has in some ways set the CR intervention questionable, because these interventions include restrictions on nutrition, but not in calories. More recently, the term dietary restriction - DR[32], which can, as mentioned above, includes various feeding regimes (alternate starvation day, intermittent starvation, change in dietary intake). A large number of studies, on the other hand, are also aimed at assessing the efficacy of natural and/or pharmacological compounds that mimic the effects of CR, i.e. CRM.

Mechanisms of calorie restriction

The cellular energy balance, essential for normal functioning of the organism, is associated with several key energy and nutritional pathways. They are also involved in the emergence of physiological dysfunction that occurs with aging. CR promotes already mentioned life-sustaining processes, and CR effects mechanisms include nutrient and energy status sensors, enzymes from the group of kinase and deacetylase, involved in post-translational modifications, signal cascades/pathways and transcription factors and coactivators necessary for the metabolic balance maintenance process. In many experimental models, several nutrient-sensing signaling pathways are described, such as insulin-like growth factor-1, IGF-1:, mechanistic Target-of-Rapamycin, mTOR; Adenosine Monophosphate-Activated Protein Kinase, AMPK; and some Sirtuin (SIRT) family members (especially SIRT-1) are cosidered essential for CR[27]. The core features of these factors are briefly listed in this section. At certain process levels there are interactions of these signal pathways, sharing many downstream targets that regulate cellular processes associated with aging, but also with CR[33].

Mechanistic (or mammalian) Target of Rapamycin is a serine/threonine protein kinase involved in various physiological processes (cell metabolism, cell survival, cell growth, autophagy), an activity that can be inhibited by the immunosuppressive drug rapamycin[34]. It is also a key regulator of cell growth and metabolism that integrates multiple signal pathways linked, among other things to cellular energy, nutrient availability and stress[35]. There are two structurally and functionally different protein mTOR-complexes: mTORC1 and mTORC2 [36] that are linked to protein synthesis, autophagy, lipid metabolism. mTORC1 is the primary modulator of protein, lipid and nucleotide synthesis, autophagy, mitochondrial metabolism and is largely regulated by the availability of nutrients, growth factors, energy, and stress levels. It seems that mTORC1 activation in higher organisms also requires the presence of insulin and IGF-1[37]. The most well-known downstream goals of mTOR are ribosomal protein S6 kinase 1 (S6K1), and eucariotic translation initiation factor, 4E-binding protein 1 (4EBP1)[38]. Although mTOR is not a direct transcription regulator, it regulates a number of transcription factors, eg. NF-κB (inflammation), peroxisome proliferator-activated receptor-κ, PPARκ (adipogenesis), transcription factor EB (TFEB, autophagy) and others[39]. mTORC2 is involved in metabolism modulation, cytoskeletal dynamics, cell survival control, but its regulation is less known[40]. Harrison et al. were the first who discovered the association of inhibitory activity of rapamycin on mTOR and prolongation of lifespan of genetic heterogeneous mice[41], as confirmed by further studies. Detailed explanation of the mechanisms of this connection still does not exist, but it is assumed to include these downstream effectors of mTOR such as S6K1 and 4E-BP[42] and, for example, autophagy activation[43], self-regeneration of some stem cells[44], resistance to different types of stress and suppression of inflammatory cytokine secretion from the senescent cell[45].

Inhibition of mTOR signaling, with rapamycin or nutritional interventions, thus protects against metabolic dysfunction, neurodegeneration and cancer, and leads to an increase in lifespan in various model organisms (yeasts, flies and C. elegans), while increased mTOR signaling is included in pathogenesis of several age-related diseases, such as type 2 diabetes mellitus and cancer[46]. Unlike other signaling pathways (AMPK and SIRT-1) as an energy sensors, mTOR activity is therefore inhibited by reducing the availability of nutrients in order to preserve energy reserves.

AMP-activated protein kinase. The cells provide energy by degradation of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and inorganic phosphate in mitochondria, whereby the rate of synthesis and ATP consumption coincides with the maintenance of the energy balance required for normal cellular processes. With this goal, during a low energy availability period, cells are rapidly replenishing ATP storage by enzymatic coupling of two ADPs, with

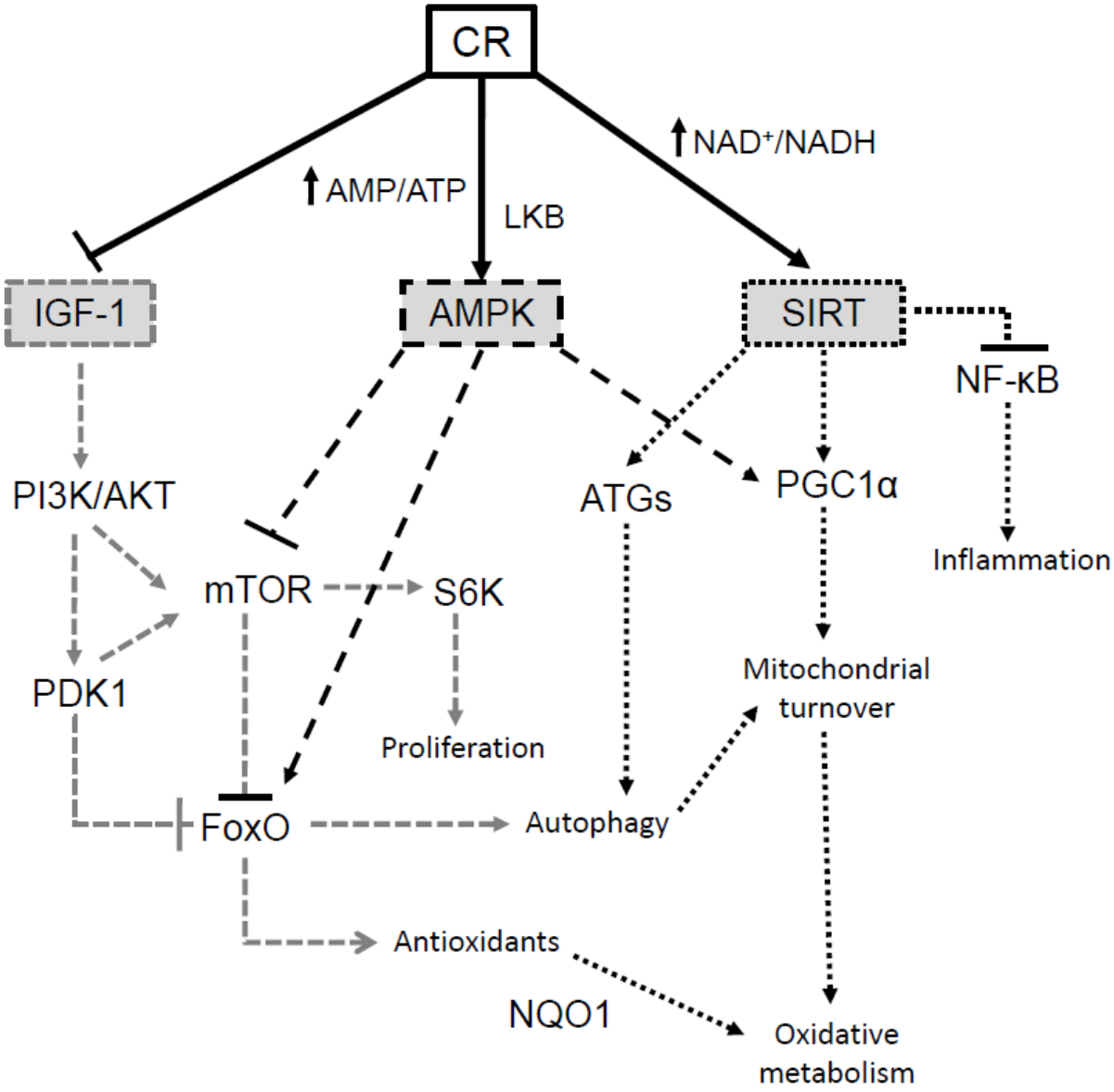

Figure 2. Postulated mechanisms of action of calorie restriction (CR), adapted according to reference (55). IGF-1 (insulin-like growth factor-1); PI3K/AKT (phosphatylinositol kinase/protein kinese B); PDK1 (phosphoinositide-dependent kinase- 1); S6K (S6 protein kinase); FoxO (forhead box protein); AMPK (AMP-dependent kinase); PGC1α (peroxisome proliferator-activated receptor α- coactivator 1-α) ; ATGs (autophagy related proteins); NQO1 (NAD(P)H dehydrogenase /quinone/-1); LKB1 (liver kinase B1); IGF-1; mTOR (mechanistic target of rapamycin): NF-αB (nuclear factor kappa B); IGF-1 patway (------); AMPK patway (- - - - -); Sirtuin patway ( )

adenosine monophosphate (AMP) as a byproduct. This compound is an important signaling molecule for sensing a low energy status of the cell by activating the AMP-activated protein kinase (AMPK) enzyme. The enzyme acts by phosphorylation of numerous downstream protein targets and transcription regulators that return the cellular energy balance[47] by activating the catabolism processes (eg. glycolysis, fatty acid oxidation) and suppressing anabolic processes (eg. protein and fatty acid synthesis). For example, AMPK regulates cellular glucose utilization, glucose transporter concentration 4, fatty acid κ-oxidation, mitochondrial biogenesis, etc.

AMPK is associated with food intake, and is the key intracellular energy sensor in cells. It is activated when the AMP/ATP ratio in the cell is increased, as occurs when cells are deprived of glucose[48]. The AMPK therefore recognizes the available amount of energy and consequently regulates other pathways important in the aging process (SIRT-1 is a positive regulator and mTOR is a negative regulator). This is a way to inhibit energy-consuming processes and activate processes that generate energy to maintain energy homeostasis. It is described that AMPK also directly phosphorylates PGC-1κ (peroxisome proliferator activated receptor-gamma coactivator 1-κ) transcriptional co-activator of nuclear receptor transcription factors. On that way it promotes lipid utilization as fuel, as well as various FoxO (Forkhead box 03) family of proteins acting as AMPK-induced autophagy mediators[49]. AMPK is a heterotrimeric protein, constructed of one κ-catalytic subunit and two regulatory κ- and κ- subunits. Under low ATP conditions, AMP or ADP binds to the regulatory κ-subunit, resulting in a conformational change that protects the activating phosphorylation of AMPK[50].

Sirtuins. In addition to regulating the aging process, sirtuins (SIRTs) are involved in CR-induced longevity. They are the enzyme family of nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylase, that affect various cellular activities. Among others, they regulate energy metabolism, stress resistance, cell fate determination, mitochondrial biogenesis, inflammation suppression, genomic stability and longevity. At the same time they interfere with different factors[51]. Sirtuins correspond to NAD+/NADH cellular fluctuations.

If there is not enough supply with nutrients, especially glucose, NAD+ will accumulate and then SIRTs will be activated. Deacetylation activity or removal of acetyl residues on numerous proteins, enzymes and transcription factors involved in key aspects of cellular physiology is related to NAD+ as a co-substrate. However, other activities of these enzymes, such as desukcinylation, demalonylation and depropionylation are also described.

Seven sirtuin enzymes members (SIRT-1 to SIRT-7) were identified among the mammals, which are localized in different cellular compartmetns[13,52]. Sirtuin-1 is largely localized in the nucleus and, to a lesser extent, in the cytoplasm. It is most closely associated with mechanisms involved in aging and longevity, by deacetylating the main regulator of biogenesis PGC-1κ and transcriptional FoxO factor[53]. FoxO proteins are capable of increasing longevity by ensuring resistance to oxidative stress, protecting protein structures and promoting lipid metabolism and autophagy[15]. Also, SIRT-1 deacetylates some glycolytic enzymes and transcription inducer hypoxia-inducible factor 1 thereby reducing the glycolysis process[54].

Insulin-like growth factor. Insulin/IGF-1 pathway (IIS) of cellular signaling is hormone-regulated, and includes insulin, insulin-like peptides, receptor substrates and numerous downstream effectors[33]. Increased serum glucose concentration after food intake stimulate secretion of insulin hormones that activates IIS signaling. This signaling pathway is also involved in modulating the aging process but also there are significant differences in this pathway depending on model organisms and mammalian species[33,35]. Activation or suppression of various dowstream effectors such as PI3/AKT/Ras (phosphatidylinositol 3-kinase/protein kinase B/Ras), mTOR, S6K and transcriptional FoxO and etc. are also involved in signaling this pathway during the CR regime[33]. CR, diet modulation, appropriate protein : carbohydrate ratio in diet, and reduced growth hormone contraction reduce the activity of IIS signaling, which is associated with health improvement and longevity of various species including human[34,56]. Due to reduced food intake during CR, signaling of this pathway is decreased, due to reduced insulin secretion and consequently the repression of FoxO factor[13]. The result is a more efficient response to stress because the transcription factor FoxO as an important target of the IIS pathway is involved in the induction of some stress response genes[33,57].

In addition to the aforementioned main regulators of signaling pathways, some factors are also described which are also, at certain levels, associated with the participants of said signaling paths during CR. These are, for example, a) neuropeptide (NP) mediated by CR-induced autophagy process [57]; b) PGC-1κ, a member of the transcription cluster family, participates in the process of mitochondrial biogenesis[58]; c) nuclear factor (erythroid-derived 2)-like 2 (Nrf2) [59], which participates in increasing the expression of mitochondrial and cellular antioxidants; d) fibroblast growth factor 21 (FGF21) as an endocrine signal associated with metabolic control, whose value changes after low protein or methionine intake[60].

The described regulators and pathways of cellular energy and sensitivity to nutrients can in particular modulate life expectancy, but as mentioned above, they are not exclusive to each other. They actually act synergistically in detecting changes in calorie/energy status in order to maintain cell homeostasis. For example, decreased availability of macronutrients is one of the most common activators for the acivation of autophagy, partly by activating AMPK and SIRT-1, and by inhibiting mTOR signaling[61]. Figure 2 shows the simplified main regulators and network of CR participants.

Under CR conditions, the ratio of ATP/AMP is changed and the AMPK is activated. Cell metabolism changes, resulting in the accumulation of NAD+, which will lead to activation of SIRT-1. An antagonistic response involves inhibition of anabolic pathways mTOR and insulin/IGF1. Downstream factors/mediators can produce redundant effects that are reflected in processes such as proliferation balanced cell growth, increased autophagy/mitophagy, improved mitochondrial biogenesis, enhanced antioxidative defense, decreased inflammation, resulting in improved healthy and prolonged lifespan.

Caloric Restriction Mimetics

In model organisms of rodents and nonhuman primates, it has been shown that diet regimes in the form of dietary adaptation such as CR, have favorable effects on health and extend life[62]. Also, results on a human samples, eg. CALERIE, Biosphere 2 Study[21], CRON Study[62], show that moderate CR improves human health and reduces various metabolic factors associated with pathogenesis of chronic diseases. Serious or severe CR slows down the accumulation of molecular damage and thus maintains key physiological functions[63]. Still existing incompatibility of the results of the long-term studies of longevity has been attributed to differently designed examinations as well as to the composition and breeding of food[64]. In addition to the CR as a strategy for beneficial effects on health and thus the aging process of model organisms, alternative life-style strategies that mimic CR, as occasional and intermitent starvation, limitation of some amino acids or protein intake, etc.[8] are described and discussion of the practicalities and effects of such approaches are ongoing.

Despite the potential benefits of CR to improve physiological functions in the aging process, most people are noncompliant to practice CR, or change nutritional routine for a long time. In other words, maintenance of CR with optimal nutrition is difficult to enforce in humans[13]. Furthermore, continuous CR represents a potential risk for older adults with normal weight who are already prone to loss of bone density and muscle mass. It is also described that reducing energy intake is aggravated by loss of skeletal muscle mass in older adults[24], and adverse reactions such as sensitivity to cold, anemia, and depression[62,65,66] are also reported in younger subjects. In restriktively fed animals, disorders in healing skin wounds[67] and susceptibility to infections[68] were observed.

In order to limit the risks of adverse effects of CR and take advantage of positive effects, over the past decade, significant attention has been paid to establishing more feasible approaches. The effects of compounds and interventions that "mimic CR or starvation" are detected and tested, that is, without real CR regime. These are: a) pharmacological and nutritional compounds, CRM and b) CR mimicking

lifestyle (behavioural) approaches to lifestyle change[69,70].
According to Ingram et al., CRMs are considered compounds or approaches/procedures, that mimic the metabolic, hormonal and physiological effects of CR, activate stress response paths that correspond to the level during CR (known under the term hormesis), enhance stress protection, produce CR-like effects on longevity, reduce the incidence of aging-related illnesses, maintain youth functions, and not reduce significantly, at least in the short run, food intake[69]. It is assumed that administration of mimetics that fulfill all or at least part of these criteria could have a positive impact on healthy life expectancy and longevity. In defining their effects, an important role will be to detect more reliable biomarkers of the aging process itself, since the existing ones still do not meet the criteria of an ideal biomarker[71].
CR mimetics are classified according to mechanisms of action such as AMPK, SIRTs (NAD-boosting compounds included herein), autophagy activators and mTOR inhibitors. Of the already discovered compounds that have the potential to imitate CR effects, some in humans have clinical use with other indications. These are, for example, rapamycin (mTOR inhibitor), metformin (AMPK activator), and polyphenolic compound - resveratrol (AMPK and SIRT-1 activators) whose various properties are also frequently tested in human populations. Other compounds referred to as CRM potential compounds are: D-glucosamine glycosaminoglycan precursor and hexokinase enzyme inhibitor used to prevent osteoarthritis[72], some antilipolylic compounds as nicotinic acid derivatives - acipimox[73], and fibrates (activate PGC1[74]; melatonin[75], SRT1-720 and SRT-2104-direct activators of SIRT-1; pegvisomant, growth hormone receptor antagonist[33]; AZD8055/INK128, a direct mTOR kinase inhibitor[33], tiazolidinediones agonists PPARs) used in diabetes therapy and obesity[13], a lipoic acid thiol compound with strong antioxidant properties[76] and others. Table 1 lists some CRMs and their mechanism of action.
As previously described, CR acts on multiple intracellular signal pathways. CR mimetics on the other hand partially induce some of these mechanisms. For example, 2DG inhibits glycolysis, thus favoring AMPK and SIRT signaling activity; metformin amplifies AMPK signaling, which indirectly leads to autophagy and amplification of the mitochondrial turnover, resveratrol increases the activity of SIRT and AMPK, etc.[77] The likelihood that some compound will stimulate all the mechanisms is small and therefore the CRM-„cocktail" effects will be tested, which would have an optimum synergistic effect on life expectancy.

Below are the main features of the most famous compounds with CRM potential.
Metformin (N,N-dimethylimidocarbonimide diamide), a member of the biguanide group, has been using for several decades in type 2 diabetes mellitus therapy since it inhibits the gluconeogenesis process and decreases insulin level[78]. It has been established that metformin has the ability to imitate most CR mechanisms and is presumed to be the main target of metformin AMPK[79]. AMPK activation leads to suppressive insulin/IGF-1 and mTOR signaling and activation of SIRT-1[27]. It is also considered that alterations in cell signaling occur secondary after inhibition of complex I mitochondrial respiratory chain resulting in ATP concentration decrease[80]. The increased AMP/ATP ratio, furthermore, activates AMPK, and consequently the transcription factors and the metabolic enzyme of said signaling pathways, in order to restore energy to the cells. Hence, they influence the processes associated with longevity such as mitochondrial turnover autophagy, stress defense, protein synthesis and inflammation[81].
The results of metformin research as CRM, are now controversial, i.e. effects on longevity are not observed in all species investigated[82]. Controversial results and the possibility of occurrence of lactic acidosis with chronic administration of metformin[83] point to caution and the need to determine optimal metformin dose for the purpose of healthy aging and longevity. The results of a human pattern study entitled "Targeting Aging with Metformin", which are underway, should form a pattern or paradigm for assessing pharmacological approaches to delay or slowdown of aging[81].
2-deoxy D-glucose, glucose analog and glycolysis inhibitor, is one of the first compounds considered as CRM[25]. It inhibits the activity of the glycolytic enzyme phosphoglucose isomerase thereby preventing the conversion of glucose-6-phosphate into fructose-6-phosphate. The

Table 1. Calorie restriction mimetics

Mimetic CR (category of compound)	Mechanisms of acting
Metformin (biguanide)	Inhibition of mitochondrial complex I; activation of PI3/AKT cascade; activation of AMPK
2-deoxy-D-glucose (glycolytic inhibitor)	Inhibition of phosphoglucose isomerase
Rapamycin (mTOR inhibitor) and rapalogs	Inhibition of mTOR leading to autophagy
Resveratrol (sirtuin activating compound)	Activation of SIRT-1
Nicotinamide riboside Spermidine (polyamine)	NAD precursor Activation of autophagy
Neuropeptide Y (neuropeptide)	Stimulation of autophagy

result is the energy supply limit in the form of ATP, and the AMP/ATP ratio increases. Next, activation of AMPK pathway is followed. Thereafter, activation of SIRTs and autophagy (84) occur. It is considered that 2DG causes delay of age-related dysfunction, and shows effects similar to CR[85]. On some organisms it has been shown that 2DG can stimulate stress response proteins and heat shock protein 70[86]. However, the attitudes of 2DG effect on life extensions differ, which is probably the result of differently designed research. A favorable effect was generally observed at lower concentrations of 2DG, while with increased concentration this compound had a cardiotoxic effect in rats[87].

Rapamycin [23,27-epoxy-3H-pyrido(2,1-c)(1,4) oxazacyclohentriacontine] is a macrolide antibiotic (also known as Sirolimus) with immunosuppressive and antiproliferative properties, most commonly used in organ transplantation[88]. The effect of promoting rapamycin's longevity was first discovered in yeast and later confirmed on numerous other organisms, while human-based examinations are still to be carried out. Rapamycin has low bioavailability, and in order to achieve higher solubility and stability, the authors emphasize the need for the development of rapamycin analogues[89]. Additional reasons for the analogs are also the immunosuppressive properties of rapamycin and the consequent possibility to develop viral and bacterial diseases. In this respect, investigations of the most effective dose of rapamycin for healthy aging and longevity are being conducted.

The mechanism by which rapamycin exerts its effect involves inhibition of mTOR signaling[90]. It is assumed that rapamycin forms a heterotrimeric complex with two proteins, FK506 binding protein (FKBP)-type peptidyl-prolyl cis-trans-isomerase and the FKBP-rapamycin-associated protein. This heterotrimer can be complexed with mTOR and thus inhibit the formation of mTORC1 complexes and consequently cell growth and proliferation. Activating of autophagy-related protein 1 (ATG1) can stimulate activation of the autophagy process[77]. Rapamycin reduces inhibition of SIRT-1 by decreasing NF-κB mediated inflammation[91].

Resveratrol (3,4,5-trihydroxystilbene) is a natural, vegetable polyphenol compound of the stilbene subgroup, with antioxidant activity being most common in grape and red wine[92]. In 2003, the in vitro NIA (National Institute for Aging) screening program confirmed the resveratrol property to activate SIRT-1, and model systems demonstrated that this activation was followed by autophagy[93]. Significant number of articles show that resveratrol prolongs the life of different species, but there are opposite opinions[94,95]. Among others, it has been described that resveratrol reduces insulin secretion, increases insulin sensitivity, decreases body fat, increases mitochondrial biogenesis and oxidative phosphorylation, increases AMPK activity, autophagy and mitophagy, and also NAD/NADH ratio, which activates SIRT-1[55]. Although the exact mechanism of action is not entirely clear, resveratrol binds to the regulatory N-terminal subunit of SIRT-1, leading to conformational change, which in turn results in increased deacetylation activity of SIRT-1[96]. Unwanted effects of resveratrol have not yet been recorded except for extremely high concentrations[97].

Spermidine. The polyamine compound, spermidine is also included in CRMs. Studies of the anti-aging effects of spermidine are still ongoing and are not quite clear, but seem to be partly related to the induction of autophagy[98]. Spermidine acts as an inhibitor of acetyltransferase EP300, endogenous autophagy inhibitor, which acts by acetylation of lysine residues within various proteins. Compounds used in traditional medicine, for example anacardic acid, curcumin, garcinol and epigallocatchin-3-gallate, also inhibit EP300[98]. In addition, spermidine exhibits various pleiotropic effects including antioxidative and anti-inflammatory activity, improvement of mitochondrial metabolic activity, and improvement of the proteostasis process. The undesirable effect of polyamines, including spermidine, is the ability to stimulate tumor progression at high concentrations[99].

CR-mimicking lifestyle (behavioral) strategies include the limitation of macro- or micronutrients (eg. protein or amino acids), periodic starvation (including, for example, feeding ad libitum 5 days a week and starvation or limiting food intake for two days), intermittent starvation (change of day of normal feeding and day of complete restriction of food, or minimum food intake) with different recommendations, i.e. restricted feeding and habitual exercise.

As it is assumed it will not be possible to find a drug that will include all pathways/mechanisms of CR without undesirable effects, the results of testing of the combination of different CRMs, CRM combinations with a specific CR level or other combinations of types will be interesting due to balancing the detrimental effects of chronic CR regime on one side, and the unwanted CRM effects on the other[89,100].

Conclusion

The aging process is an inevitable process that is additionally adversely affected by the development of age-related diseases. In the area of geroscience, great efforts have been made to detect the causes and mechanisms of this process in order to establish appropriate genetic or nutritional/pharmacological interventions for the purpose of healthy aging and longevity. This does not seem easy, since the mechanisms are networked, ie., there are mutual activating or suppressing actions.

The CR has now proved to be a promising intervention in achieving that goal. CR promotes life-prolonging processes such as autophagy, mitochondrial biogenesis, and ATP production. Although the ideal calorie intake is currently unknown and is likely to be different for each person, the results of CR research on various model systems and types have provided insights into the main signaling pathways of healthy aging and longevity.

Some of these pathways have become the main goals or candidates for CRM development that could be used for the purpose of healthier aging or postponement of aging-related illnesses. Besides examinations of individual, potential CRM compounds, but also investigations with their combinations and combinations of CRM and other imitation CR strategies are performed. Alternative CR strategies occasional starvation, modulation of nutritional input/balanced input

of macronutrients, and other effects to be explored in future research. It is assumed that different strategies will also have different effects on complex pathway signaling participants. It is to be expected, that the real direction will be given interdisciplinary research that will define the most optimal type of strategy. Undoubtedly, the implementation of omics-based approaches such as genomics, transcriptomics, metabolomics and proteomics will be of great help.

Discussion about aging as a) normal/partially normal or natural or b) partial pathological process is ongoing, and the latest developments in gerontology reveal the molecular link between aging and pathological conditions. However, many open questions remain about the aging process at the molecular level. Generally, it is necessary to try, if possible, to define more clearly processes that induce aging, ie "natural" aging and age-related diseases. The application of CRM on a human beings for the purpose of achieving longevity, will only be enabled and justified after researches has been carried out on a number of non-human primate models, which for now have a small number, and after considering ethical, economic and social implications.

Author contributions:

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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