Maria Inês Branco Abegão

Caloric Restriction Mimetics: The Next Generation of Anti-Aging Treatments

Monografia realizada no âmbito da unidade de Estágio Curricular do Mestrado Integrado em Ciências Farmacêuticas, orientada pela Professora Doutora Claúdia Margarida Gonçalves Cavadas e apresentada à Faculdade de Farmácia da Universidade de Coimbra

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Coimbra, 14 de setembro de 2016.

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(Maria Inês Branco Abegão)

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To all my family for all the support and care, constantly present

Particularly to my sibling and my parents for being there forever, for always and no matter what.

"If we wait until we're ready, we'll be waiting for the rest of our lives."

Lemony Snicket

Contents

Abbreviations

AMPK, AMP-activated protein Kinase;

BMI, Body Mass Index;

CALERIE, Comprehensive Assessment of Long term Effects of Reducing Intake of Energy;

CRON, Caloric Restriction with Optimal Nutrition;

FoxO, Forkhead box protein O;

GH, Growth hormone;

IGF-1, Insulin-like Growth Factor 1;

NPY, Neuropeptide Y;

PGC-1α, Peroxime-proliferator Activated Receptor γ Coactivator-1;

PPAR, Peroxime proliferator-activated receptor;

ROS, Reactive Oxygen Species;

SIRT-1, Sirtuin 1;

Resumo

O envelhecimento é um dos principais fatores de risco de várias doenças do mundo moderno. O processo de envelhecer pode ser influenciado por aspetos internos e externos. A restrição calórica pode retardar o envelhecimento reduzindo doenças associadas a este e aumentando a esperança de vida. Diversos estudos mostram que os efeitos benéficos da restrição calórica parecem ser mediados por vários mecanismos, tais como a sinalização insulina/IGF-1, a estimulação da autofagia, PCG - 1α, sirtuinas, AMPK e Neuropeptideo Y.

A dificuldade em manter uma dieta de restrição calórica com nutrição ideal (CRON) encorajou a busca de miméticos da restrição calórica, fármacos capazes de mimetizar os benefícios da restrição calórica e aumentar a esperança de vida. Neste trabalho iremos descrever compostos que têm sido descritos como potenciais miméticos da restrição calórica induzindo mecanismos semelhantes a esta. Os miméticos da restrição calórica aqui descritos incluem a 2-desoxi-D-glucose (inibidor glicolítico), Metformina (biguanida), Pegvisomant e Octreoside (antagonista do IGF-I), Resveratrol (agonista da SIRT-1) e o Neuropeptido Y.

Palavras-chave: Restrição calórica; Envelhecimento; Miméticos da restrição calórica; autofagia; PGC-1α; sirtuinas; AMPK; insulina; Hormona do Crescimento/IGF-1

Abstract

Aging is a major risk factor for numerous diseases in modern world. The process of aging can be influenced by intern and extern aspects. It was demonstrated that caloric restriction can delay aging reducing age-related diseases and increasing life span. Caloric restriction's increase in life span seems to be mediated by several mechanisms such as insulin/IGF-1 signalling, autophagy stimulation, PCG-1α, sirtuins, AMPK and Neuropeptide Y.

The difficulty in maintaining a caloric restriction with optimal nutrition (CRON) diet encouraged the search for caloric restriction mimetics, drugs capable of mimicking caloric restriction benefits and increase life span. In the present work we describe several compounds that have emerged as potential caloric restriction mimetics inducing overlapping mechanisms to caloric restriction. Among these are included 2-deoxy-D-glucose (glycolytic inhibitor), Metformin (biguanide), Pegvisomant and Octreoside (IGF-1 antagonist), Resveratrol (SIRT-1 agonist) and Neuropeptide Y.

Keywords: caloric restriction; aging; caloric restriction mimetics; autophagy; PGC-1α; sirtuins; AMPK; insulin; Growth Hormone/IGF-1

1. Introduction

Aging is a natural process of cell senescence and deterioration which can diminish cell resistance to stress, loss of function and, therefore, age-related diseases (1,2).

Several studies show that caloric restriction increase life span of different laboratory models and animals, such as yeast, nematodes, spiders, flies, fishes, rodents, dogs and also primates (1,3). The first time it was shown that reducing the total amount of calories ingested could increase life span on rats was in 1930 by McCay (4, 5, 3).

This article will focus on two major issues: firstly, caloric restriction mechanisms and its benefits to humans; thus, possible caloric restriction mimetics, how they may act and what evidence there is on their efficacy in increasing life span.

2. Caloric Restriction

Caloric restriction consists on reducing the total amount of calories ingested by an organism in a well-balanced diet. This means diminish the energy intake by 20-60% below *ad libitum* for a long period of time without create any nutrient malnutrition (6).

The mechanisms by which caloric restriction extends life span is believed to be by a reduction on age-related decline and age-related diseases even though it is not completely clear how this delay occurs (7). When long-term caloric restriction occurs, there are physiologic and metabolic changes such as reduced resting metabolic rate, decreased core body temperature and reduced inflammatory signalling. This might support the idea that caloric restriction is the best way to improve health and longevity but there are also some possible changes not so beneficial such as weight loss over the safety parameters (BMI≤18,5kg/m2), reduced fertility, reduced bone density, anaemia, etc (6). Therefore, this issue requires further research.

2.1. Mechanisms underlying the beneficial effects of Caloric restriction

There are several measurable outcomes underlying the beneficial effects of Caloric restriction, such as reactive oxygen species (ROS) decrease, body temperature decrease, resting metabolic rate reduction, circulating T3 and TNF-α decrease (6,8).

As for the molecular mechanisms, there are several signalling pathways that seem to be part of the overall caloric restriction outcome on improving health and life span even though may present some controversy. These mechanisms include Insulin/IGF signalling, autophagy stimulation, sirtuin activation, among others (1).

2.1.1. Insulin/IGF signalling

After ingesting food, glucose levels in the blood rise and insulin is secreted. When the connection insulin-insulin receptor happens, there are numerous downstream factors (PI3K, AKt, Ras) activated that repress the transcription factor FoxO (1). This transcription factor is composed by several proteins, FoxO1, FoxO3a, FoxO4, and FoxO6, and it regulates stress response genes. Caloric restriction naturally decreases this signalling, since if there is less food ingested, less insulin will be secreted and, consequently, less repression of FoxO resulting in a more effective response to stress (1,7).

Growth hormone when released, can induce the production of Insulin-like Growth Factor 1 (IGF-1). This protein is very similar to insulin so it can activate insulin signalling pathway. It is known that low levels of IGF-1 can increase life span since its increases insulin sensitivity (2). People with Laron-type Dwarfism have a mutation in growth hormone receptor and present a very low concentration of IGF-1 leading to a great resistance to diabetes and cancer, two of the most common age-related diseases. Following this logic, we can assume that, when it comes to growth hormone or insulin, the less the merrier (9).

However, total depletion of growth hormone is not beneficial and leads to death at birth in mice (2). The key seems to be a reduction of function without total depletion. Tazearslan C. et al (2) found that centenarians present a combination of several rare SNPs in IGF-1 which support the importance of this factor and Insulin signalling pathway in human longevity.

In another investigation it was studied the connection between growth hormone and caloric restriction. Even though not all pathways are coincidence, many of the caloric restriction mechanisms are related to growth hormone and insulin signalling, particularly the ones associated to gluconeogenesis (10). Mice knockout to growth hormone receptor might present a better stress resistance which is also much related to aging and can reduce the risk of senescence and inflammatory disorders (10).

These reasons seem to support the strong connection between insulin and growth hormone with life span through this pathway. If this relation is real, we can search for caloric restriction mimetics that interfere with this pathway either through insulin or growth hormone.

2.1.2. Autophagy Stimulation

Another possible mechanism of action of caloric restriction is the stimulation of autophagy. Autophagy is an intracellular process that breaks down macromolecules to use their components (11). This turnover is of extreme importance to the cell and its dysfunction is associated to accumulation of damaged proteins and, consequently, the aggravation of age-related diseases (11,12). It is described that autophagy decreases with aging which explains why we are more willing to illness as we grow older (12).

Caloric restriction is one of the physiological triggers of autophagy acting through the depletion of acetyl coenzyme A (AcCoA) (13). EP300 is an acetyltransferase that transfers acetyl groups from AcCoA to autophagy core proteins inhibiting autophagy. The depletion of AcCoA caused by caloric restriction can lead to the deacetylation of these proteins followed

by AMPK activation and mTORC1 inhibition. MTORC1 is the main suppressor of autophagy (13).

The mTOR (mammalian target of Rapamycin) regulates cell growth and senescence and is composed by the mTOR complex 1 and the mTOR complex 2. The mTOR complex 1 is sensitive to Rapamycin once it has the regulatory-associated protein of mTOR and it is the one responsible for inhibit autophagy and promote protein synthesis. The mTOR complex 2, on the other hand, is insensitive to Rapamycin, regulates the protein kinase and affects cells metabolism and survival (14).

MTOR inhibits autophagy and stimulates protein synthesis. When cell cycle is blocked because growth is no longer possible, TOR effects lead to cellular loss of function and senescence (11). Caloric restriction can supress mTORC1 through activation of AMPK increasing autophagy and, consequently, delaying aging (11).

2.1.3. Peroxisome-Proliferator Activated Receptor γ Coactivator-1(PGC-1α)

PGC-1α is a member of the peroxisome-proliferator activated receptor γ coactivator-1(PGC-1) family of transcriptional coactivators and promotes the expression of numerous genes involved in mitochondrial and fatty acid metabolism (1). It is known that PGC-1α increases mitochondrial biogenesis and respiration rate plus the uptake of substrates in order to increase energy production (15). With aging, mitochondrial activity slows down which causes muscle decay, neurodegenerative disorders and general metabolic diseases. PGC-1α can retard muscle loss and protect against this age-induced decline. PGC-1α can also diminish the switch between lipid to carbohydrate metabolism that characterises some common heart diseases (16) and activate the mobilization of triglycerides from white adipose tissue to stimulate gluconeogenesis (1).

Calorie restriction induces mitochondrial biogenesis mediated by PGC-1α. To increase PGC-1α, caloric restriction can act by two mechanisms: activation of SIRT-1, which deacetylates PGC-1α, and reduction of glycogen synthase kinase 3β (GSK3β), a PGC-1α repressor (1). PCG-1α can mediate most of caloric restriction effects on muscles such as mitochondrial biogenesis, alteration on adipose tissue metabolism and prevention of the metabolic shift in the myocardium (1,16).

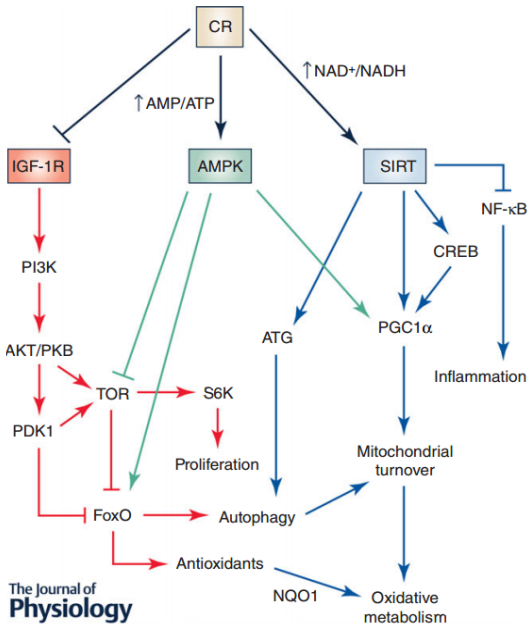

Figure 1. Complex interaction of proliferative and protective mechanisms in the CR prolongevity effect in organisms Nearly all the organisms studied to date have shown similar mechanisms in response to CR. Basically, CR induces mechanisms involved in energy efficacy and inhibits mechanisms involved in less efficient energy consumption and proliferation. Thus, CR inhibits IGF-I-dependent signalling which activates TOR and protein synthesis and inhibits FoxO, blocking antioxidant expression and autophagy. On the other hand, the balance between AMP and ATP and NAD+ and NADH serves as a signal to activate AMPK and sirtuins, respectively. These nutrient sensors and regulators block the signal dependent on IGF-I and TOR at the same time as they activate mechanisms to enhance more efficient energy production through oxidative metabolism and mechanisms to enhance cell protection through antioxidants and organelle turnover through autophagy. At the same time, CR, through sirtuins, can block inflammation mediators. This regulation occurs at different levels and many mediators can produce a redundant response such as autophagy activation by blocking TOR-dependent FoxO inhibition or by inducing the activity of autophagy-related (ATG) proteins.
Adapted from (18).

2.1.4. Sirtuins

Sirtuins are a class of NAD+-dependent deacetylases and can either act as NAD+-dependent deacetylases, mono-ADP-ribosyl transferases or have both activities (1,17). In mammals, there are 7 sirtuins with different locations: SIRT-1 is found mostly in the nucleus but also in the cytoplasm. SIRT-2 is found exclusively in the cytoplasm. SIRT-3, SIRT-4 and SIRT5 are in the mitochondria and SIRT-6 and SIRT-7 are predominantly in the nucleus (17).

Sirtuins control several processes such as energy metabolism, stress resistance, cell survival and longevity interfering with different factors.

SIRT-1 is the most studied sirtuin so far and it is strongly linked to the mechanisms related to aging and longevity through deacetylation of PGC-1α and FoxO (17). When deacetylated in the skeletal muscle, PCG-1α promotes mitochondrial biogenesis (1,19), activation of FoxO leads to autophagy and FoxO-dependent induction of stress response genes (1). Besides, SIRT-1 deacetylates glycolytic enzymes and the transcription inducer, HIF-1α,

shutting down glycolytic metabolism (19). This sirtuin targets are present in most organs that start to deteriorate with age (17).

Numerous studies have proven that sirtuins mediate effects of caloric restriction. In yeast, the ablation of Sir2, the homolog of sirtuins, inhibits life span effect of caloric restriction (19). The same happens in metazoans (20). Mice under caloric restriction regimen presented higher levels of SIRT-1, SIRT-3 and SIRT-5. In humans under caloric restriction regimen, higher levels of SIRT-1 were also induced (17). Besides, mice knockout to SIRT-1 do not live longer under similar conditions (17). Transgenic mice overexpressing SIRT-1 are relatively immune to several diseases such as diabetes, bone loss, neurodegenerative diseases, etc, similar to caloric restriction profile, which confirms SIRT-1 importance to prevent age-related diseases (17).

2.1.5. AMP-activated Protein Kinase (AMPK)

AMPK is intrinsically bound to food intake and, in the case of energy shortage (low AMP/ATP ratio), it triggers several mechanisms such as autophagy (1).

AMPK is intimately related to SIRT-1 since they both act as energy sensors and can regulate coincident mechanisms (21). AMPK induction of mitochondrial fatty acid oxidation varies NAD+ concentration activating SIRT-1 (21). SIRT-1 can per se activate AMPK by increasing its deacetylation in the liver (1,21).

In addition, AMPK phosphorylates PCG-1α and FoxO making them accessible to SIRT-1 deacetylation (1). These mechanisms support the theory of an energy sensing network between SIRT-1 and AMPK that regulates energy expenditure essential to caloric restriction effects (21).

Worms and flies overexpressing AMPK lived longer supporting this enzyme beneficial effect on longevity (7). In *C. elegans* caloric restriction effects were proven to be AMPK-dependent (7). Despite this evidences, it is still unclear its benefits in mammals.

2.1.6. Neuropeptide Y

Most of the mechanisms activated by caloric restriction mentioned above result in autophagy. In a different approach to this issue, it was evaluated the effect of one of the major neuropeptides in the hypothalamus, Neuropeptide Y (NPY). NPY may have a major influence on caloric restriction by stimulating the hypothalamus to increase autophagy (12). With aging, levels of Neuropeptide Y (NPY) in brain decrease and so does autophagy (22). Therefore, it seems likely that this neuropeptide plays an important role on aging (12).

There are four G protein-coupled receptors subtypes present in the hypothalamus, Y_1, Y_2, Y_4 and Y_5 Receptors (14). Recent studies show that the activation of Y_1, Y_2, or Y_5 receptors stimulates autophagy in hypothalamic neuros and also in cortical neurons (12, 23) to autophagy pathways. Y_2 receptor, however, appears to be more relevant to other cells in the hypothalamus than neurons (12,24). As for Y_1 and Y_5, they both activate PKA leading to the unravelling of a cascade that ends in autophagy. Y_1 uses a PI3K-dependant pathway to stimulate AKT leading to autophagy. Y_5, on the other hand, activates PI3K, MAPK1/3, and PKA pathways even though it is not described as a PI3K-dependant pathway (24).

Recently it has been demonstrated that NPY mediates caloric restriction induced autophagy (23). In rats subjected to caloric restriction, cortical neurons show increased autophagy and NPY. To better understand NPY role in caloric restriction induced autophagy, it were used NPY receptors antagonists. No caloric restriction induced autophagy was observed suggesting that this effect is NPY-dependent (23).

2.2. Effects of caloric restriction in humans

Different studies have been done in order to evaluate the beneficial effects of caloric restriction in humans and to understand whether these effects are comparable with the ones found in animals (4).

The CALERIE Study Group (Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy) is the first randomized controlled trial of caloric restriction in humans (4). The first phase consisted on a 6 to 12-month 25% caloric restriction in overweight people assessed by three research centers (Tufts University, Pennington Biomedical Research Center, and Washington University) (4). In this Phase 1, results show that caloric restriction decreases body weight and adipose tissue (4). It was also described a reduction in body core temperature, energy expenditure, T3, fasting insulin and an upgrading in insulin sensitivity (5). In caloric restriction subjects it was also reported a significant decrease on DNA damage markers, similar to those described to animals (5).

These promising results lead to the CALERIE Phase 2 that took 2 years and included people with BMIs between 22 and 28kg/m^2. The main purpose this time was to study caloric restriction effects after the stabilization of weight loss (25,6). The results obtained were rather similar in terms of decrease in body weight, body fat and body core temperature. These decreases were more notorious during the first year of the study suggesting an adaptation to the diet after that time. Energy expenditure, TNF-α and T3 were also diminished, suggesting an improved control of thyroid axis and inflammation (6). Moreover,

triglycerides and cholesterol diminish, insulin sensitivity increased, and blood pressure decreased. These results suggest a decrease in cardiovascular risk factors, the most common age-related diseases in the modern world (6).

One major concern associated to caloric restriction is the risks that might imply to restrict so extremely our source of energy (26). In order to understand this, the CALERIE study evaluated other factors such as quality of life, mood, sleep, etc (26). There were no negative influences on mood, sleep or sexual life reassuring that caloric restriction can be implemented in non-obese population. In fact, some of these parameters show an increase suggesting that caloric restriction could actually ameliorate quality of life (26). There were, however, some risks associated with this treatment and some subjects had to be evaluated. One of the major worries was bone mineral density loss which can lead to osteoporosis, more common in women, and eventually, fractures (6). Anemia was also a big issue and two of the participants develop an excessive decrease in the hematocrit and had to be withdrawn from the study. Depression was also more common in caloric restriction group (6,27).

In another study, Wittea A. et al. (28) tested the effect of caloric restriction on memory in an elderly population. The study was conducted with fifty subjects over 3 months. The age mean was around 60 years and there were three groups: 30% calorie restriction; 20% relative increase in unsaturated fatty acids; and control. In the end of the three months, verbal memory was evaluated and the caloric restriction group presented the biggest increase (around 20%) as well as a general decrease in risk factors such as Protein-C reactive and fasting insulin. Other groups did not present significant changes (28).

In a different study, Meyer T. et al. (29) suggested that caloric restriction could improve diastolic function. To study this influence, twenty-five subjects were evaluated after a medium of 6.5 years of caloric restriction. Even though systolic function did not show significant differences, compared to the control group, diastolic function was improved in the caloric restriction group similar to systolic functions described to younger persons. Blood pressure and systemic inflammation were also lower resulting in a decrease on overall risk of cardiovascular disease (29).

3. Caloric Restriction Mimetics

Despite all the beneficial outcomes given to caloric restriction, to maintain a Caloric Restriction with Optimal Nutrition (CRON) diet is hardly feasible in humans (1). Thus, to find a drug that acts as a caloric restriction mimetic and retard aging is very pertinent. A caloric restriction mimetic drug should be able to mimic caloric restriction beneficial effects (1). This opens the door to a whole new family of anti-aging medicines acting through the same mechanisms that underlie caloric restriction effects and putatively increase life expectancy and diminish age-related disorders (14).

The definition of caloric restriction mimetics has been changing over the last decades and is still a controversial subject. Some authors consider a widen concept while others consider a narrow one (14). Some authors consider anorectic agents or substances that inhibit nutrient absorption as caloric restriction mimetics (14). Others consider the molecules capable of mimic caloric restriction mechanisms inside the cell whether it induces a metabolic, hormonal or physiological response without interfering with food intake (1). This drug effects on cells could be putatively converted in life span extension (1).

Since caloric restriction affects many intracellular pathways, it is rather unlikely to find a compound able to induce all the mechanisms. Instead, caloric restriction mimetics induce partially some of these mechanisms (1).

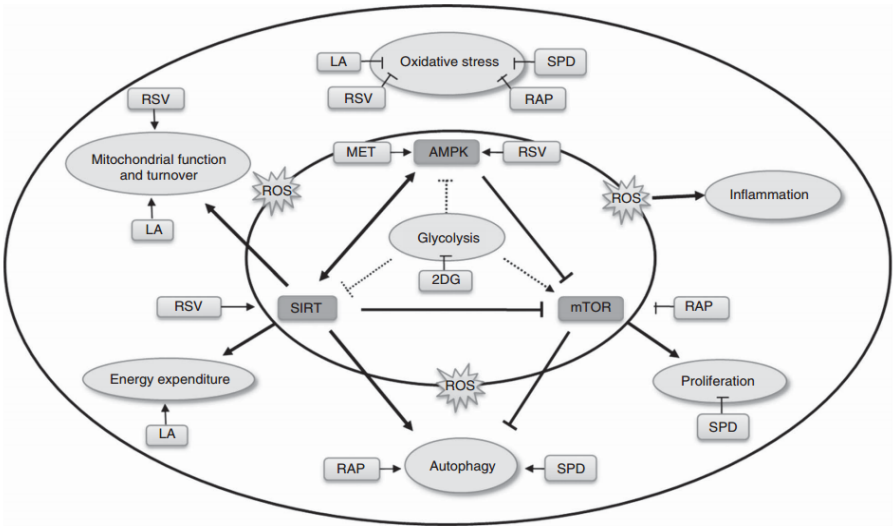

Figure 3. Schematic overview of the suggested molecular targets of the energy restriction mimetic candidate substances 2-deoxy-D-glucose (2DG), metformin (MET), rapamycin (RAP), resveratrol (RSV), spermidine (SPD) and lipoic acid (LA). 2DG inhibits the central process of glycolysis, thereby favouring the activities of AMP-activated protein kinase (AMPK) and sirtuin (SIRT). MET increases AMPK activity, indirectly leading to increased autophagy and mitochondrial turnover. RAP inhibits mammalian target of rapamycin (mTOR) signalling, thereby favouring autophagy and inhibiting proliferative processes. In addition to its antioxidant capacity, RSV is thought to increase SIRT and AMPK activity. SPD might up-regulate the antioxidant response, enhance autophagy and decrease proliferation. LA might improve mitochondrial function, increase energy expenditure and reduce oxidative stress. ROS, reactive oxygen species. Adapted from (30).

3.1. The 2-deoxy-D-glucose

One of the first potential caloric restriction mimetics found is 2-deoxy-D-glucose (14). This glycolytic inhibitor can prevent phosphoglucose isomerase which avoids glucose-6-phosphate transformation to fructose-6-phosphate. This inhibition stops this energy pathway causing an energy depletion. The lack of ATP induces AMPK which ends up activating autophagy (31,32).

When research to find a caloric restriction mimetic began, 2-deoxy-D-glucose seemed like a promising candidate by targeting glucose metabolism and inhibit energy supply. In 1998, Lane et al (33) set a study in which male rats were supplemented with different doses of 2-deoxy-D-glucose. It was revealed a decrease in fasting insulin and body core temperature, effects similar to those obtained with calorie restriction, but it was also discovered that the higher dose of 400-450mg/kg was toxic. Despite this discovery, there were additional studies that reinforced this drug potential as a caloric restriction mimetic. It was demonstrated that 2-deoxy-D-glucose can increase stress response by stimulating stress response proteins, heat shock protein-70 and glutamate responsive protein-78 (34,35). Glutamate receptors are involved in several neurodegenerative disorders associated with aging such as Huntington's disease so 2-deoxy-D-glucose protection against glutamate excitotoxicity can delay these disorders (34).

Later, Wan et al (36) observed that rats fed with 2-deoxy-D-glucose were more resistance to stress than the control group accumulating evidence on life span extension properties of this drug. Kang and Hwang (37), on the other hand, claimed that 2-deoxy-D-glucose effect could not increase life span and it could, in fact, induce some transcriptional factors contrary to those induced by calorie restriction. These conflicting results led to a general precaution on declaring this drug safe.

3.2. Metformin

Since insulin signalling is one of the proposed mechanisms of action to caloric restriction, drugs with impact on this pathway are obvious candidates as caloric restriction mimetics. Metformin is a biguanide that has been used to treat diabetes for the last 70 years and it is known its capacity of improving glycolytic metabolism in these patients (38). Now it is believed that its effects on healthy individuals might be beneficial as well, once it can mimic some of the caloric restriction effects and extend life span (38,39). Indeed, some of the aspects accomplished with caloric restriction such as reduced plasma insulin, reduced

gluconeogenesis or reduced glucose absorption in the gastrointestinal tract, can be also obtained with Metformin (14,38).

Moreover, Metformin inhibits mitochondrial respiratory-chain complex I that results in depletion of energy, as ATP (40). This increase of ADP/ATP ratio will activate AMPK which triggers transcription factors and metabolic enzymes to restore energy in the cell. Among these enzymes are phosphoenolpyruvate carboxykinase and glucose-6-phosphatase that supress gluconeogenesis in the liver leading to fatty acid oxidation and glucose uptake (40,41). Fatty acid oxidation is stimulated because gluconeogenesis is inhibited so, the cell needs to produce energy from other sources. Glucose uptake is stimulated by the translocation of GLUT4 to the plasma membrane which enhances peripheral glucose utilization by an insulin independent pathway (14). AMPK high concentrations will also activate SIRT-1, inhibiting mTOR complex 2 and, furthermore, inhibiting gluconeogenesis (14).

Despite all these effects, the activation of insulin/IGF1 receptor pathway follows to the PI3K cascade and ends in the activation of FoxO transcription factors. Onken and Driscoll (38) studied the possibility of life extending properties of Metformin not being entirely due to its effect on insulin (38). Mutant *Caenorhabditis elegans* (*C. elegans)* lacking FoxO were given Metformin in three doses and life span was measured. The authors observed a life span extension of 36% in mutant strain concluding that Metformin can increase life span without insulin signalling (38). These results were reinforced by a second experiment were mutant nematodes lacking the PI3 kinase were given Metformin and then compared to a control group. In this case life extension achieved was 43% (38).

It was then evaluated the possibility of Metformin activate the same pathways that caloric restriction. Metformin was given to *C. elegans* under a caloric restriction profile. In this strain, it was not observed any life span extension. In fact, the higher doses were toxic suggesting that the mechanisms by which Metformin acts were already activated and it excessively activation could cause negative outcomes similar to those obtained by extreme caloric restriction (38).

In a study which compared several caloric restriction mimetics effect with caloric restriction on life span of yeast, Metformin fail to enhance life span (42). The same happened in the *Drosophila melanogaster* (*D. melanogaster*) model. Even though Metformin reduced lipid stores and activated AMPK, life span did not change (43).

In a different study, Dhabi et al. (44) tried to find caloric restriction mimetics through microarrays screening of the transcriptional profiles in mice. From all the screened

molecules, Metformin appeared to exhibit the best performance activating 75% genes related to long-term caloric restriction and 92% genes related to short-term caloric restriction (44).
All these recent studies strongly suggest that Metformin is able to mimic most of the mechanisms driven by caloric restriction and extend life span even though not all species might share this effect. In fact, Metformin's effects on life span seem to be specie, dose and even sex-dependent (45). The dose particularly, appears to be crucial to its effect since Metformin can either increase, decrease or even do not change life span (46). Therefore, further investigations are required to confirm these data as well as stablish the right dose for these beneficial effects. Barzilai (47) has now proposed a clinical trial to study aging as a treatable condition and plans on proving that Metformin, despite its use in diabetes type 2, can be the first anti-aging drug.

3.3. Pegvisomant and Octreotide

The reduction of growth hormone (GH) and, consequently, IGF-1 can extend life span (14). The decrease of GH can diminish insulin/IGF-1 signalling leading to a higher insulin sensitivity. Following this point of view, to increase life span, we can putatively act through inhibition of GH release or inhibition of GH receptor signalling pathway (14).
Pegvisomant is an antagonist of GH receptor prescribed in cases of acromegaly, a disease caused by an exaggerated release of GH. This drug, via growth hormone's antagonism can reduce levels of IGF-1 which, consequently, reduce the risk of diabetes and cancer, two of the most common age-related disorders in current world (1,14).
Octreotide is also a GH receptor antagonist and some of its side effects are common to those of Pegvisomant making it a possible caloric restriction mimetic as well. Even though Pegvisomant might have a more favourable effect on glucose metabolism, some small investigations suggest that Octreotide can likewise play an important role in age-related diseases (48). In type 1 diabetic patients under insulin treatment, Octreotide ameliorated blood glucose control and (48) it seemed to improve memory in Alzheimer's diagnosed old adults (49).
These studies, however, do not allow any conclusion since most of them implicated too few individuals. It still remains unclear whether these two drugs can be used has anti-aging agents. Despite their effects on glucose metabolism, Pegvisomant and Octreoside present some serious and unpleasant side effects like dizziness, vomiting and diarrhoea that can compromise their safety as a caloric restriction mimetic. Moreover, their excessive cost makes it very hard to study and develop an anti-aging drug, particularly Pegvisomant (1).

To sum up, we now know that growth hormone and IGF-1 are strongly connected to diabetes and cancer but modulation of this axis in a pharmacologic way is yet too far. These pathways might be more complex than we thought at first and further research is required to understand completely this mechanism and generate a potential caloric restriction mimetic based on this axis (14).

3.4. Resveratrol and others Sirtuins Activators

Caloric restriction activates Sirtuins, therefore sirtuin-activating compounds are a probable candidate as caloric restriction mimetics. SIRT-1 is the most studied sirtuin and its known effects on increasing human longevity (17). Several molecules have the capacity of activating SIRT-1 but Resveratrol is the most potent known so far (50).

Resveratrol is a polyphenol extracted from some plants such as the grape *Vitis vinifera* and has already risen many debates on its effect on human health (51). This molecule seems to promote mitochondrial biogenesis through deacetylation and activation of PGC-1α in a SIRT-1 dependent way (52).

This polyphenol has increased life span in many different species such as yeast, *C. elegans* and *D. melanogaster* through activation of Sir2, SIRT-1's homolog in mammals (17). The discovery of this mechanism suggested that resveratrol might act through the same pathway as caloric restriction. Therefore, several studies have been carried out to figure whether this molecule can be used as an anti-aging drug or not by simulating energy restriction in the cell (3).

Resveratrol increases *C. elegans* and *D. melanogaster* life span (14). This effect, however, was not observed when *D. melanogaster* where already under a restrict diet or had a mutation in Sir2 gene (14). This strongly suggest that Resveratrol activates Sir2, an activation also achievable with calorie restriction. The activation of Sir2 is similar in both treatments but cannot be additive (14). This assumption though, must be taken very carefully for many contradictory results have been obtained on life span effects of Resveratrol in invertebrates (1).

There are also some issues that may compromise this drug possible success as caloric restriction mimetic. For example, Resveratrol oral bioavailability is very low. This could explain why there are such controversial results. The administration route is not the same in all studies. It is also alleged that its linkage to the fluorophore Fluor de Lys used in some studies to reveal binding and acetylation of proteins has induced misinterpreted results on previous investigations (1).

Baur et al (53) showed that Resveratrol protects against insulin resistant in mice. Szkudelski (54) confirmed this result proving that Resveratrol inhibits insulin secretion by acting directly in pancreatic cells in live rats. In wild-type mice Resveratrol increases the metabolic rate and reduces fat mass increasing insulin sensitivity and glucose tolerance. This effect was not observed in AMPK-deficient animals (1) These claims AMPK influence in Resveratrol effects. Some authors suggest that Resveratrol effects are mainly due to a primary activation of AMPK instead of SIRT-1 and that this kinase then activates PGC-1α leading to its beneficial actions (1). Nathan Price et al. (52) performed a study with adult mice in which Resveratrol administration induced mitochondrial biogenesis and protection against metabolic decline *in vivo*, outcomes not observed in SIRT-1 knockout mice (52). Conversely to these results, *in vitro* tests showed that the higher concentrations of Resveratrol could, in fact, activate AMPK in a SIRT-1independent mechanism and cause mitochondrial dysfunction but, for some reason, none of these effects were observed *in vivo* (52). It still remains unknown which of these mediators, SIRT-1 or AMPK, is the primary target of Resveratrol and which doses are safe for its caloric restriction mimetic effect (52).

Some recent studies showed a general improvement in blood glucose and lipids in obese adults and in diabetic adults but this was not observed in normal weight individuals. In older subjects, Resveratrol seemed to improve memory performance (14,55). Others studies show Resveratrol capacity to improve health biomarkers such as endothelial function, left ventricle diastolic function and insulin sensitivity but only on subjects with pre-existence metabolic disorders (55).

Others polyphenols have been studied as potential sirtuins activators such as quercetin, catechins and anthocyanins but additional investigations are required to tell if any of these possible candidates can, in fact, be a promising caloric restriction mimetic (1).

There are already some start-ups companies dedicated to develop and produce sirtuin activators. This area is still emerging but there is no reason to doubt that not very long from now there will be an anti-aging drug programmed for sirtuin activation (14).

3.5. Rapamycin and other mTOR inhibitors

TOR receptor is associated to aging since it regulates autophagy as well as other important pathways such as S6 kinase. It seems logic that Rapamycin becomes a prime target as caloric restriction mimetic. After all, mTOR takes its name from Mammalian Target of Rapamycin (30). This macrolide is used since the 80s as an antibiotic for inhibiting mTOR receptor and reducing cell growth and proliferation. It is not completely understood how this inhibition

occurs even though some studies suggest that Rapamycin can bind to two proteins and that this heterotrimer then complexes with mTOR preventing the formation of mTOR Complex 1 (30). These properties have led to its later use as an immunosuppressant under the name of Sirolimus to prevent organ rejection after a transplant. Now, it has been described that Rapamycin can, not only prevent tumors, but also induce regression of pre-existence ones (1). Cancer is one of the most common cause of death in the modern world so, if we prevent it, it is rational to think that life expectancy would rise.

In order to evaluate whether Rapamycin can exert some of the caloric restriction effects, Yu et all (56) analysed the transcriptome and metabolome of male mice in four groups: mice fed ad libitum (control group); mice treated with 14ppm of Rapamycin; mice subjected to 40% calorie restriction; mice treated with Rapamycin and subjected to 40% calorie restriction. After 6 months of treatment, groups subjected to Rapamycin and caloric restriction alone presented similar results in terms of autophagy, lipolysis and life span extension (56). Furthermore, when the two treatments were associated, effects on gene expression were higher. This suggests that, even though, some effects are overlapping, not all caloric restriction mechanisms are activated by Rapamycin and vice-versa. This hypothesis is supported by the fact that long treatments with Rapamycin induce glucose intolerance, an outcome completely opposite to the one obtained with caloric restriction (56). The reflective analysis of the transcriptome and metabolome revealed that both treatments increase mice life span by mTOR inhibition, activation of autophagy and suppression of cell senescence. However, Rapamycin increased insulin resistance. As for lipid metabolism, both treatments were successful in inducing lipolysis and reducing fat storage but in Rapamycin treatment, since β-oxidation is not activated and there is no lack of nutrients, this might cause excessive blood lipids which end up promoting the insulin insensitivity mentioned in this treatment alone (56). In addition to these side effects on glycolytic metabolism, Rapamycin can also influence immunity since it is used as an imunossupressant. These side effects are not irreversible (1) and seemed to be avoided or remarkably diminished by intermittent treatments (57).

Moreover, rapamycin inhibition on mTOR has been shown extend life span by reduction of S6 kinase on so many species that hopes are high on that it can exert this effects on humans as well (1). This drug has been used in humans for many years now so much research has already been made on its safety leaving its effectiveness as a life span extensor the only unanswered question to solve (57).

Studies on Rapamycin's effect on human life span are scarce. Zoya N. Demidenko et al. showed that Rapamycin can delay and prevent human cell senesce in vitro even though it could not reverse it. However, it is yet unknown whether this effect can be observed *in vivo* (11).

Rapamycin low bioavailability demanded the urge to analogues with enhanced water solubility and stability. In the last few years, some analogues have risen in the market as cancer therapies. In spite of its side effects, many authors strongly believe that Rapamycin can, in fact, delay aging in a revolutionary way and eventually it will become the most effective anti-aging treatment (57). It remains unconfirmed Blagosklonny's prediction: "Will Rapamycin become the cornerstone of anti-aging therapy in our lifetime?" (58).

3.6. Neuropeptide Y

Recent studies show that Neuropeptide Y (NPY) stimulates autophagy in neurons (12). Moreover, the blockage of NPY receptors inhibit the stimulatory effect of caloric restriction on autophagy stimulation (24). Moreover, when NPY is injected directly in the hypothalamus results in lower blood glucose, hyperphagia, decreased core body temperature, fertility decrease. Some of these effects are similar to caloric restriction. It was also described an enhanced resistance to stress and increase life span in rats (12).

Human studies on NPY administration are scarce but it its effects as an endogenous molecule have been studied. Female centenarians have presented higher levels of this neuropeptide in their blood which supports its relation with longevity (12). NPY is also neuroprotective and its lower concentrations are associated to neurodegenerative diseases, most of them age-related diseases as well (12,24).

These evidences strongly suggest that NPY could be a promising caloric restriction mimetic and act as an anti-aging agent (12,24), however, further investigations are required to know its feasibility for it presents a serious limitation, the administration route is not practicable for most people (59).

Table 1 Caloric restriction mimetics

Compound	Category	Mechanism
2-Deoxy-D-glucose	Glycolytic Inhibitor	Inhibition of phosphoglucose isomerase
Metformin	Biguanide	Inhibition of mitochondrial complex 1; activation of P13 cascade
Pegvisomant and Octreoside	GH/IGF-1 Antagonists	Reduction of GH/IGF-1 signalling
Resveratrol	Sirtuin Activating Compounds (STACS)	Activation of SIRT-1
Rapamycin	TOR Inhibitor	Inhibition of mTOR leading to autophagy
Neuropeptide Y	Neuropeptide	Stimulation of autophagy
D-glucosamine	Amino sugar	Inhibition of hexokinase
Spermidine	Polyamines	Activation of autophagy
Thiazolidinediones	PPARγ Agonists	Activation of PPARγ transcription
Lipoic Acid	Antioxidants	Reduction of ROS

3.7. Other emerging candidates

This is an expanding field so there are several potential candidates as caloric restriction mimetics: D-glucosamine (14), Spermidine (14), Thiazolidinediones (30) and Lipoic acid (1).

D-glucosamine is a precursor of glycosaminoglycans, the main component of joint cartilage, so it has been used to treat and prevent osteoarthritis (14). D-glucosamine has emerged has a possible candidate once it was described to mimic a low-calorie diet (30). In recent studies, this component extended life span in nematodes and aged mice. In this last case, mice revealed decreased blood glucose without effect on food intake similar to caloric restriction effects (14). D-glucosamine can act has an inhibitor of hexokinase in glycolysis when phosphorylated which may be a possible mechanism for its caloric restriction effects. It has also been described its effects on Akt/FoxO/mTOR pathway stimulating autophagy in cartilage (60). The fact that this drug has been used for 50 years now provides a powerful advantage since its safety profile is already known making it a potential candidate in the race of caloric restriction mimetics (14).

One of the most recent candidate caloric restriction mimetic are polyamines, particularly **spermidine** (14). Polyamines are essential to several cellular processes that control pH, cellular volume and membrane potential maintaining cell integrity and cell survival. Spermidine is a precursor of other polyamines and in humans its production decreases with age. In centenarians, though, there have been described high levels of this molecule supporting its importance to aging (14). Unlike some polyamines, spermidine seems to protect against oxidative stress and it has been demonstrated to increase life span in yeast,

worms, flies, aged mice and human cells (30). Even though its mechanism is not well stablished it is believed to act through the activation of autophagy in a SIRT-1-independent pathways. Not many investigations have been made on this molecule yet since it is a very recent subject (14).

Agonists for peroxisome proliferator activated receptors (PPAR) have also been suggested as prospective caloric restriction mimetics. **Thiazolidinediones**, a class of PPAR γ agonists, are already used to treat diabetes and obesity. In caloric restriction conditions, PCG-1α is increased so, if we activate its co-activator with PPARγ, we can mimic somewhat these conditions. By activating PPARγ transcription, these drugs can increase insulin sensitivity and ameliorate lipid metabolism (15,30). A recent study has suggested that thiazolidinediones can activated AMPK in a PPARγ-independent way. Further studies are required to confirm this hypothesis (1).

Another proposed caloric restriction mimetic is **Lipoic acid.** This molecule is a potent thiol antioxidant and it seems to protect against oxidative damage (1). Lipoic acid has increased life span in invertebrates but studies on mammals have not demonstrated this effect yet. Microarray studies, however, show that this drug can stimulate gene expression of some pathways related to caloric restriction (1). Although studies with Lipoic acid are still very rudimentary, the impact of oxidative damage in aging and the benefits of antioxidants to prevent this are well known issues raising hopes for this drug as a possible restriction mimetic.

4. Conclusion

Since it has been showed by numerous studies that caloric restriction increases life span of most species (1,19), a whole new concept of anti-aging therapy through caloric restriction mimetics appear.

This approach changes the way we see aging. In fact, instead of treating each disease such as diabetes or hypertension the scientific community should focuses on finding strategies to delay aging itself preventing diseases that have aging as main risk factor (14).

Despite all the development done so far, it is not clear if a drug will ever be able to mimic caloric restriction effects without risks to human health (14). Is not only about increasing life span, is about increasing the life span with health (healthy life span). There are some promising molecules that may have the potential to extend life span and exert their effects through several mechanisms similar to caloric restriction.

It may not be possible to find a drug capable of mimic all caloric restrictions pathways without side effects so there will probably be the need to associate two or more drugs (1). Some of these hypothesis are already being tested such as a combined therapy with Rapamycin and Metformin, to obtain all the benefits from Rapamycin treatment missing the side effect on glucose metabolism (56).

Some of these drug are already in the market and there are cases where their benefits on aging are already being proclaimed like Resveratrol, for example, which can lead to its uncontrolled use without the correct scientific support (14).

It is of great importance that further well stablished and organised studies are concluded on Rapamycin's effect on life span in order to avoid misinterpretations of results. There is also the need to stablish greater biomarkers to measure and evaluate longevity and disease resistance in humans (61).

5. What does the future holds?

If all these promises come true, what can we expect from the future? Will we be able to dribble most common age-related disorders of our times? It appears so. Over the last century many diseases have been eradicate which increase life span. The major difference in this case is that we will no longer treat a specific disease. We will treat aging instead (47).

How far are we from this reality? Most of these caloric restriction mimetics are already under clinical trials or are drugs already on the market with other indications (1). All this evolution is happening pretty fast so we can believe that in a few years from now there will be caloric restriction mimetics sold as revolutionary anti-aging drugs in pharmacies (14).

Will Mankind achieve the promised fountain of youth? It is rather unlikely that a caloric restriction mimetic can reverse a bad diet and lifestyle (51). People probably will never be able to live without following healthy habits if they want to live longer. In fact, that it has never been the purpose of caloric restriction mimetics (51). These drugs are created to take advantage of caloric restriction benefits. They are not a magic potion that can increase longevity regardless of our lifestyle. That, unfortunately, will remain a faraway dream.

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