# The Potentials of Calorie Restriction on Lifespan and Health – A Literature Review

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## Abstract

Calorie restriction has been found to prolong lifespan and prevents age-associated diseases in several species. What causes these beneficial outcomes is still a topic of research. Whereas some study's point to the ratio of macronutrients rather than the caloric intake as regulator of the beneficial effects, other studies do point to added life- extending potential of calorie restriction over protein restriction alone. This literature review addresses these contrasting findings, questionable methodologies and pitfalls in translating findings from model-organisms to humans and gives insight into future research-needs.

## Introduction

According to the Global Health Observatory (GHO), the global average life expectancy increased by 5.5 years between the years 2000-2016 (1). The prognosis is that by 2050 the number of Americans, aging 85 years and over will have tripled (2). Along with the increase of life-expectancy comes the increasing incidence of age-related diseases, including cardiovascular diseases (most common cause of death in older adults) (3, 4), cancer (the second leading cause of death in older adults) (2, 5), hypertension (6), osteoarthritis (4), diabetes mellitus (predicted to increase by more than 400% by 2050) (7), osteoporosis (particularly among older women) (8), cognitive aging (e.g. dementia) (9), and in almost two-thirds of the cases, a combination of two or more of those chronic conditions (10, 11). So although life expectancy increases, the health span or ''optimal longevity'' lacks behind. Current research is focussing on extending this optimal longevity by investigating how physiological functions can be preserved and optimized throughout life so that the morbid and disabled phase onsets in a shorter period later in life.

It was first reported in 1935 by McCay and colleagues that limiting daily food intake in rats extends their lifespan (12). This feeding regimen is called calorie- (CR) or dietary (DR) restriction. It is defined by the National Institute on Aging (NIA) as ''reducing average daily caloric intake below what is typical or habitual, without malnutrition or deprivation of essential nutrients''. Since the first report, researchers have heavily tested this intervention-method on life extension on a variety of species including *Saccharomyces cerevisiae* (yeast) (13), *Drosophila melanogaster* (fruit flies) (14), *Caenorhabditis elegans* (nematodes) (15), a broad range of laboratory mouse and rat phenotypes, wild-caught mice (16), and *Macaca mulatta* (rhesus monkeys) (17). These studies point to different nutrient-responsive pathways, including the mammalian target of rapamycin (mTOR) as effectors of CR (18). In translation to humans, the Comprehensive Assessment of Long term Effects of Reducing Intake of Energy (CALERIE)-study, reported promising outcomes (19).

Despite the proclaimed potentials of CR on achieving optimal longevity, there are contrasting findings, questionable methodologies and pitfalls in translating findings on model-organisms to humans. This literature review provides an overview of the current understandings of the mechanisms on which CR plays a role, experimental approaches with their strengths and weaknesses, and gives insight into future research-needs.

# 1. The Dietary Aspects of Calorie Restriction

As mentioned earlier, CR is no more than reducing the caloric intake below what is typical or habitual, commonly referred to as *ad libitum* (AL). Malnutrition is thereby prevented. However, this is quite a broad description of CR and does not explain how CR is achieved regarding dietary composition, onset (stage of life), and protocol. This section provides an overview of different approaches to induce CR in model-organisms.

## 1.1. Dietary composition

Different organisms have different nutritional demands. But even within species, the dietary composition varies greatly between studies. Part of the reason why this is still occurring is that the role of optimal dietary composition for promoting lifespan extension remains largely unknown at present day.

In most cases, diets that are given to laboratory animals are either composed of natural or purified ingredients. Natural diets vary from batch to batch due to nutritional fluctuations caused by harvest location and growing season. Purified diets overcome this disadvantage and are very consistent in their nutritional composition, ruling out cofounding experimental variations. However, natural diets generally are a more complete source of nutrition and are better in mimicking a typical diet compared to purified nutrient diets.

In an effort to gain insight into the nutritional needs for optimal lifespan, an approach called the 'Geometric framework' was developed originally in insects. State space models yielded optimal levels for nutrients on growth and metabolism (20), and was later used to dissect out contrasting impacts of nutrition and aging in invertebrates (21). The results in the latter study implicated that the effects of CR were not per se caused by the diminished calorie input but rather involved complex interactions between the macronutrients that lead to the desired effects, at least in invertebrates. The translation to vertebrates (mice) was later made by Solon-Biet *et al*, 2014 who claimed that the longevity effect was driven by the protein to carbohydrate ratio (22). This fed the debate on whether it is CR or protein restriction that yields the desired effects that has been going on for almost a century. However, a more recent study by Speakman *et* al, 2016 that extracted data from the literature dating back to the 1930s, shows different life-extending effects of CR and protein restriction (23). The authors state that reducing protein levels in rodents by 80% increases the median lifespan by about 15% while reducing calories by 40% increases median lifespan by on average twice as much. In other words, twice as much gain of lifetime can be achieved with 40% CR as opposed to 80% protein restriction. Speakman and colleagues ascribe the difference in outcome to Solon-Biet's approach in maintaining CR by diluting the diet with indigestible cellulose, resulting in the ingestion of fewer calories, but twice as much total mass of food. This approach may result in no *bona fide* CR-effect since a potential key component of the response to CR is the stimulation of the hunger signalling pathways in the brain (24-26). Therefore, the Speakman-study states that a *bona fide* CR-study should not try to mimic AL-conditions, but should allow for the sensation of hunger to occur.

Another example of how differences in dietary composition can lead to different outcomes is the comparison of the Wisconsin National Primate Research Center (WNPRC)-study (27) with the National Institute of Aging (NIA)-study (28). Both were 20-year-lasting longitudinal studies on Rhesus monkeys. The WNPRC-study showed improved survival associated with 30% (moderate) CR initiated in adult Rhesus monkeys (figure 1). These findings were not confirmed in the NIA-study. One of the differences between the two studies was, indeed, diet composition. The diet formulation from the NIA-study was based on natural ingredients whereas the WNPRC study used

mixed purified components. One component of importance that was notably different was carbohydrate. Although similar in total mass, the NIA study used ground wheat and corn whereas the WNPRC study used corn starch and sucrose (28.5% sucrose compared to 3.9% in the NIA study). This high level of sucrose may increase the risk of developing type II diabetes decreasing life expectancy (29). Although the NIA-study failed to report lifespan-extension upon CR, they did confirm that CR can prevent tumour progression in Rhesus monkeys.

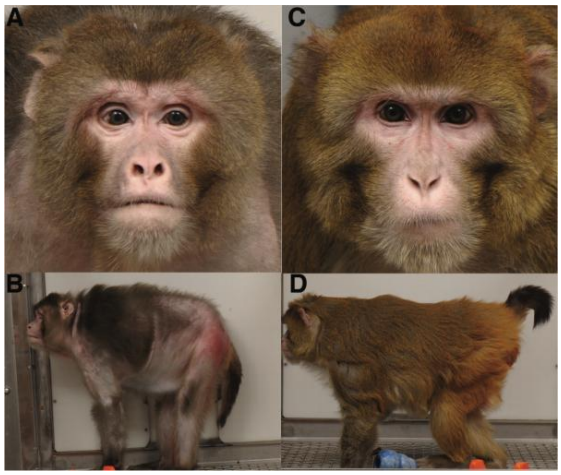


**Figure 1: Rhesus monkey appearance in old age (27.6 years) in the WNPRC study.** A and B were AL-fed. C and D were on CR (adapted from Colman *et al,* 2009 (27)).

Most studies on CR with laboratory animals compare their experimental animals to an AL-fed group. Uncontrolled AL overfeeding of excessive calories to rodents has been recognized as one of the most important determinant errors to the variability and poor reproducibility. There is a highly significant correlation between AL food consumption and obesity and body weight, and low 2-yr survival in rodents (76). Comparing a CR-group to an AL-group can lead to misleading interpretations of the results. Therefore, a moderate CR is a more appropriate method of dietary control. The NIA- study on Rhesus monkeys took this factor into account by regulating the proportioning of food for the control group and thereby possibly imposing a slight CR in the control monkeys. Also, the WNPRC- monkeys weighed more than the corresponding NIA- monkeys. Possibly the NIA- monkeys were in a more optimal weight - range. These factors may have been reasons why this study failed to report a life- extending effect of CR.

In summary, when investigating the effects of CR on longevity, vertebrate studies on mice and non-human primates show that it is best to attempt to mimic a natural diet, composed of all necessary (macro) nutrients, without repressing the hunger-sensation with fillers. This may be different if the study aims to investigate a specific pathway induced by CR. Furthermore, a true control-group is also slightly dietary restricted because true AL will lead to overfeeding which influences the outcomes. Finally, it is of importance to maintain palatability since an unpalatable diet may result in a self-imposed dietary DR (30).

### 1.1.1. Carbohydrates

Energy-wise, carbohydrates are the largest contributors in a diet. As already mentioned in section 1.1, sucrose can induce type II diabetes. A long-term study on rats showed a 10% reduction of lifespan in those consuming a sucrose-based diet compared to those fed corn-starch (31). The type of sugar that is consumed impacts physiological outcomes, even under CR-conditions (32). Simple carbohydrates such as glucose, fructose, sucrose, and lactose are notorious for rising blood glucose levels quickly, resulting in frequent peaks in blood-insulin levels. Insulin activates the mTOR-pathway which reduces lifespan (section 5). In the long term, hyperglycemia can reduce insulin-sensitivity and result in the development of type II diabetes (33). Complex carbohydrates such as starch keep the body fuelled for an extended period of time. This decreases the frequency and intensity of blood-insulin-peaks. Furthermore, complex carbohydrates help postponing hunger which can eventually help losing weight instead of gaining weight. Another benefit of complex carbohydrates is that they can lower LDL cholesterol and thereby reduce the risk of developing cardiovascular disease (34).

### 1.1.2. Protein

Work throughout the 2000s have demonstrated that at least part of the CR-effect on rodent lifespan is due to reduced protein intake, specifically branched-chain amino acids (BCAA) (23). These BCAAs (L-leucine, L-isoleucine, and L-valine ) activate cellular responses associated with aging via the mTOR pathway (section 5) (35). BCAA's have been found to increase protein intake, whereas all other amino acids reduce appetite. Proteins play a major role in muscle homeostasis. A high-protein diet can repress the progression of sarcopenia, an age-related and potentially life-threatening condition in which skeletal muscle mass and strength decline over time (36). This indicates that there may be an optimal dose of amino acids in a diet that provides the desired health benefits without inducing the hyperactivation of pathways resulting in detrimental effects. This is currently a heavily investigated topic of research (22).

The source of protein was for a long time considered a key factor for health-outcome. Animal protein fed to rabbits was associated with atherosclerosis and plaque formation whereas when rabbits were fed soy protein, they did not develop vascular lesions (37). A major limitation of the experimental trials is that only one type of animal protein (typically casein) was compared with only one type of vegetable protein (typically soy).

A prospective study on ± 30.000 post-menopausal women in 2005 revealed that long-term adherence to high-protein diets, without discrimination toward protein source, may have potentially adverse health consequences (38). A bigger player in the health-effects of protein consumption may not be the protein source but rather the method of preparation. Frying, barbecuing, and broiling of meats increases the exposure to highly carcinogenic heterocyclic aromatic amines which can induce several types of cancer (39-41).

### 1.1.3. Fat

Like carbohydrates and protein, dietary fat sources can also impact health outcomes in both desired and undesired ways. Body fat has been reported to have pro-inflammatory effects and increases the risk for developing cardiovascular disease, rheumatoid arthritis, osteoarthritis, periodontal disease, and cancer (42, 43). However, high-fat diets that are high in plant-based unsaturated fatty acids are associated with increased bone strength and density, lower body weight, and extended lifespan (16, 43). Long living mammals have small amounts of unsaturated fatty acids in their cell-membranes. Depletion in fatty acids results in increased cellular protection against lipid peroxidation. CR in mice prevents the accumulation of long chain glycosphingolipids in the kidneys during aging (44).

Considering the evidence discussed here regarding the potential effects of the various macronutrients assembled to make a diet, it comes to no surprise that at present, the ideal ratios of carbohydrate to protein to fat remain unknown. Nonetheless, evidence suggests that low-protein, high-carbohydrate diets are associated with improved health in humans (45, 46), and extended lifespan in mice (22), although CR has more health potential than solely protein restriction, as discussed in paragraph 1.1.

## 2. Age of Onset of CR Yields Different Outcomes

CR can be imposed on animals at different stages of life, bringing different desired and/or undesired effects. This section addresses the implications of young-, adult/middle-aged-, and old-onset CR.

In general, young-onset CR is imposed on animals in early life but post-weaning. For mice, this stage is typically between three and six weeks (47).

For Rhesus monkeys, this is between 40-175 weeks (f) and 40-287 weeks (m) (48). In this stage of life, the animals are still developing both physically and mentally. In both mice and nonhuman primates, CR has been shown to slow skeletal growth and delay maturation (26, 49, 50). Furthermore, CR is found not to be uniformly tolerated in young nonhuman primates resulting in a higher mortality rate (51). In general, the consensus is that CR in this stage of life is not a useful approach to manage long-term risk at the expense of potential short-term negative outcomes (52). Early adulthood seems to be the best period in the life-cycle of murine animals to start with CR (53). The same is true for Rhesus monkeys (28). Early adulthood CR in mice has shown that although concentrations of reproductive steroids are reduced, they decline less rapidly with age than AL mice, such that later in life, levels may actually remain at higher concentrations (16). Old onset CR, (>20 months) in mice, demonstrates less lifespan benefits compared to earlier in life although reports have shown that old-onset CR can reverse the aging process of the hart (54), and also slow down the age-dependent loss of cognitive and motor skills (55). Most studies however, report increased mortality in most commonly used strains when starting CR only in late-life (47).

## 3. Species- and Sex Differences

The effects of CR on health and survival are not universal. Amongst others, species- and sex-dependent effects interact with the CR-outcome. Also, the use of certain model-organisms brings practical challenges. These aspects are discussed in this section.

The Rhesus monkey, which shares ± 93% genetic identity with humans (56), serves as a representative model for human aging. Like humans, they develop age-related diseases, including diabetes, sarcopenia, osteoporosis, and cognitive decline (57, 58). Furthermore, they exhibit similar feeding patterns and sleeping behaviour (51). Their human-like characteristics also bring some weaknesses when using nonhuman primates. As they can become up to 40 years of age, studying the course of aging takes up to decades to yield results. Furthermore, sexual dimorphism has been reported in Rhesus monkeys where females lacked response in bodyweight upon CR compared to the control-group, suggesting sex-specific connection between nutrition and disease risk (51).

When investigating (potential) mechanisms of CR, short-lived species including yeast (13), *Drosophila* (14), nematodes (15) and rodents have several advantages over the use of long-lived species. Their genetic make-up is usually well characterized which allows to control these genes and measure alterations in expression upon CR. Genetic manipulation makes it possible to study (parts of) pathways while leaving the rest unaffected. These model-organisms usually have a short life-cycle which makes studying them more money and time-efficient.

Yeast are a commonly used model organism to study CR. Usually CR is achieved by limiting the availability of glucose. 98% CR is not uncommon for these studies. Despite the extensive use of this model-organism, a 2014 systematic review reported that the effect of CR on lifespan has been routinely overestimated in yeast due to the use of short-lived experimental controls. The inability of CR to robustly extend lifespan suggests that calories alone do not modulate the lifespan of this important model organism (59). In *Drosophila* similar contradictions are found. A typical diet for *Drosophila* consists of yeast as a source of protein and carbohydrate (glucose). In general, CR may shorten lifespan in *Drosophila*, rather than prolonging it (60). However, a positive effect on survival may be obtained by a decreased protein: carbohydrate ratio (60). Proteins are essential in female *Drosophila* for egg production, but male flies require little to no protein during adulthood.

Especially in males, overconsumption of protein may have toxic effects (61).

The nutrition of *C. elegans* in an experimental setting commonly relies on a bacteria- based diet. A 10-fold decrease of bacterial density have been reported to result in 60% increase in lifespan (62). Higher dilutions can extend the lifespan to up to 150% (63). A weakness of the dietary makeup is that the food-source contains all (macro)nutrients. Therefore, CR-effects can only be generalized to overall food consumption, rather than dissecting individual effects of each macronutrient (44). the *C elegans* model is primarily appropriate for explaining the causality of general nutritional phenotypes (e.g. fat content). The use of *C. elegans* model for questions in human nutrition is rather limited, because the metabolism as well as the nutrient intake differs markedly between nematodes and humans (64).

It is of high importance to always keep in mind that findings in model-organisms are not per se translatable to the human organism. Laboratory strains typically are optimized for research purposes which can influence the outcome. This has been demonstrated by Austad *et al* 2003 who studied third-generation wild-caught male mice (*Mus musculus*) and failed to demonstrate the typical CR effect because according to the authors, most studies on CR have utilized animals that have adapted over many generations of laboratory life and have been selected on favoured traits such as rapid growth, early maturity, larger body size, and a high reproductive rate (16). Austad and colleagues demonstrated that laboratory mice eat about 20% more than wild mice under *ad libitum* (AL) conditions (65).

One example of physiological differences between rodents and humans is the relationship between adiposity and mortality. In rodents, the adipose-levels typically gradually increase during their lifetime (66). Studies have shown that males are more vulnerable to increased adiposity (followed by a greater inflammatory response) and glucoregulatory dysfunction than females (67). In humans and nonhuman primates, typically the adipose-content starts to drop when the final stage of life is reached (51, 68). The WNPRC- study on nonhuman primates demonstrates a significant relationship between adiposity and survival in the female cohort (figure 2), indicating a sex-specific relationship between body fat and morbidity.

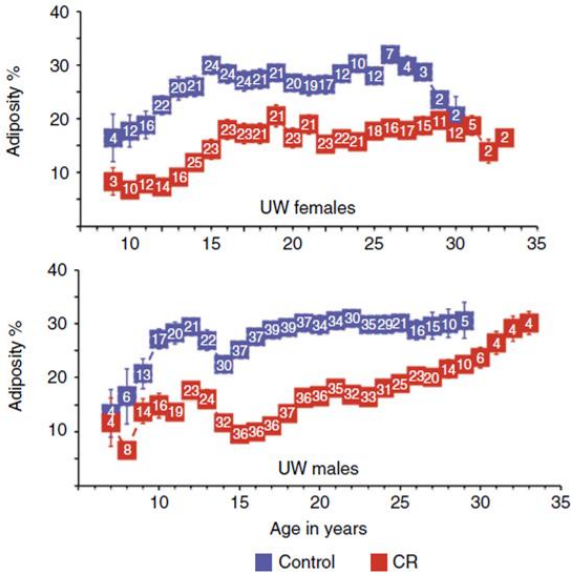


**Figure 2: Adiposity data of Rhesus monkeys from the WNPRC-study grouped by age.** CR= 70% of AL. Digits shown in white within the boxes are the numbers of individual animals contributing to each data point. Adapted from Colman *et al,* 2009 (27).

## 4. CR therapies

CR can be imposed periodically or continuously and can be mild or more severe. These aspects of CR are discussed in this section.

CR can be roughly divided into two categories: limited daily feeding (mostly used in animal experiments) and intermittent fasting. With limited daily feeding, the subject receives a daily caloric percentage of what is considered normal. Typically, the proportion of food that is given daily, is all there is. So if the subject eats all in a shorter period of time, it will be fasted during the rest of the day (69).

Intermittent fasting is described as a feeding protocol in which periods of access to food are

alternated with periods of a complete absence of food. Examples of intermittent fasting include regimens in which access to food is altered between 24 hours of AL-access to food and 24 hours without access. A different version is a time-restricted access to food within a period of the day (70). This method of CR is not new as different cultural groups e.g., the Islamic culture, self-impose a yearly 30-day intermittent fasting-period in which no food is consumed between dawn and sunset. A major potential danger with intermittent fasting is that many species, including humans, will overeat when the food becomes available, resulting in no net change in caloric intake compared to control groups (71).

Because the minimum degree of restriction that yields the maximum benefit has not been identified, most studies base their CR-regimen on other publications. The NIA and WNPRC-studies both used a consecutive 30% CR. For mice, 40-60% CR is typically used, but some studies go as far as 80% (16, 72). It is important to consider the age, begin-weight, and strain in order to select a CR-regimen. Younger mice and some strains are more sensitive to undernutrition than others (16).

The CALERIE-study was the first randomized controlled study on human CR. The study imposed a 2-year 25% CR in ±150 nonobese healthy participants aged 25-45 years. Body fat contents were significantly reduced upon CR as well as cardiometabolic risk factors including LDL- and HDL cholesterol, insulin, and blood pressure. Also, quality of life and psychological outcomes were reported. Participants of the experimental group were less vulnerable to binge eating (against expectations), felt less concern about body size and shape, had better physical functioning which ameliorated quality of life, and their cognitive performances remained unchanged (19). Although 25% CR is seen as a moderate form op CR, it has shown to positively affect life-extending factors without the occurrence of negative (mental) side-effects, at least for the duration of 24 months.

## 5. mTOR Manipulation Plays a Key Role in the CR-Effect

The mTOR pathway plays an essential role in the mammalian metabolism and physiology (35). In this section, the normal functions of mTOR are discussed as well as its effects on lifespan and roles in disease.

mTOR is a kinase that links with other proteins and serves as a core component of two distinct protein complexes, mTOR complex 1 and mTOR complex 2. Each complex regulates different cellular processes. Upon signalling from insulin/ insulin-like growth factor 1 (IGF-1), amino acids (BCAA's), and other factors, mTORC1 regulates cell growth, cell survival, cell proliferation, protein synthesis, autophagy, and transcription. mTORC2 promotes the activation of insulin receptors and IGF-1 receptors (73, 74).

Clinical studies have shown that high levels of BCAAs correlate with insulin resistance (75). This occurs via a negative feedback loop, displayed in figure 4. In brief, amino acids (BCAAs) activate mTORC1 via two pathways: Rag-Ragulator and Vps34/PLD1. Once activated, mTORC1 phosphorylates Insulin receptor substrate 1 (IRS1) and Growth factor receptor-bound protein 10 (Grb10) to block insulin signalling. Overloaded nutrients induce sustained mTORC1 activity that causes insulin resistance. A lower concentration of BCAA's in the circulatory system will eventually increase the insulin sensitivity and still activate the mTOR pathway although in a lesser extent (73). Interestingly, protein restriction has been proven to have the potential to reduce the level of the circulating IGF-1 and to increase the level of the IGF-1 binding proteins. Therefore, protein restriction both directly (via amino acid- signalling) and indirectly (via insulin-signalling) decreases mTOR- activity. Studies have shown decreased mTOR activity increases lifespan in several short-lived organisms (22). The mTOR pathway becomes dysregulated in a variety of human diseases, such as diabetes (76), obesity, and a multitude of cancers (77).

mTOR overactivity significantly contributes to the maintenance and development of tumours, mainly because of its effect on protein synthesis and the inhibition of autophagy.

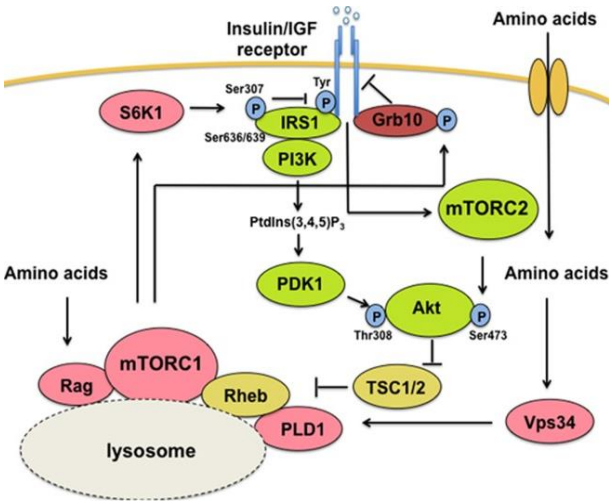


**Figure 4: Mechanisms in which mTORC1 senses amino acids and induces insulin resistance**. Amino acids signal to mTOR via two parallel pathways: Vps34/PLD1 and Rag-Ragulator. mTORC1 is activated when the pathways result in the binding of mTORC1 to the lysosome. Insulin stimulates PI3K which promotes phosphorylation of Akt by PDK1. Insulin also activates mTORC2 which phosphorylates Akt at a different position. Akt phosphorylates TSC2 to block its GTPase activating protein (GAP) activity for Rheb, thereby inhibiting mTORC1. Activated mTORC1/S6K1 phosphorylates IRS1 and Grb10 to block insulin signalling (negative feedback loop). Overloading amino-acids or insulin/IGF-1 induce sustained mTORC1 activity that causes insulin resistance. Adapted from Yoon *et al*. 2017 (73).

In normal conditions, autophagy is the main player in the removal of damaged proteins from the cells. This process becomes inhibited by overactive mTOR (figure 4). CR increases the AMP/ATP ratio which activates AMP-activated kinase (AMPK). AMPK initiates autophagy by forming a complex with proteins derived from autophagy-related genes (ATG), a set of approximately twenty evolutionarily conserved genes required for autophagy. Also, AMPK directly inhibits the mTOR-pathway which inhibits the formation of this complex. In the process of aging mTOR activity increases while autophagy decreases, resulting in an accumulation of damaged proteins (78). Studies have demonstrated that decreased mTOR activity increases lifespan in several short-lived organisms (22).

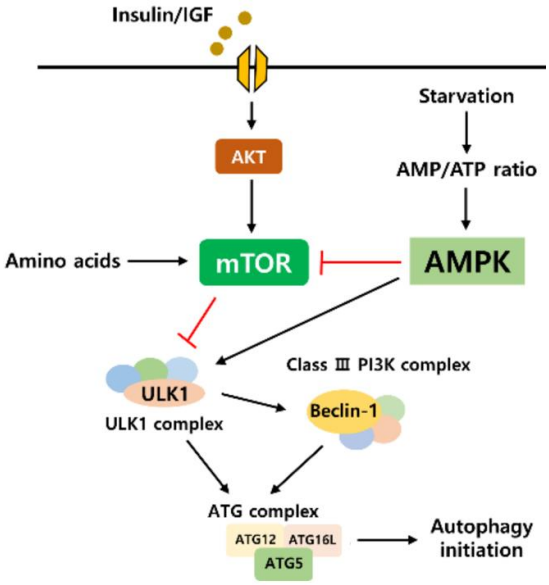


**Figure 5: Mechanisms in which autophagy becomes activated or inhibited.** Overactivation of the mTOR pathway results in the inhibition of the formation of the ATG complex, eventually blocking autophagy initiation with an accumulation of damaged proteins as a result. CR activates AMPK which blocks the mTOR pathway and initiates the formation of the ATG complex. Here autophagy is both directly and indirectly stimulated. Adapted from Chung *et al,* 2019 (78).

## 6. Discussion

Since the first report on the effects of CR in model-organisms in 1935, researchers have extensively investigated this non-pharmacological, non-genetic mechanism to extend life span and health span, with inconclusive outcomes.

It appears that the type of model-organism of use plays a role in the CR-effect. For example, CR has shown the potential of life-extension in different murine strains but not in all, possibly because the strains that do not respond to CR have been selected for favourable traits in which longevity pathways are already working optimally. Also, sexual dimorphism regarding CR has been discussed. In *D melanogaster* nutritional protein is only required for egg-laying females. In fact, nutritional protein quickly becomes toxic in males. Sexual dimorphism has also been reported in Rhesus monkeys where females lacked response in bodyweight upon CR compared to the control group, suggesting a sex-specific connection between nutrition and disease risk.

At present, the scientific community is still debating over whether the CR effects comes from the restriction of calories or the change in macronutrient-ratios. Studies have shown that altering protein intake, without restriction for calories does have life-extending effects in mice, but less so than in CR. The macronutrients of which a typical diet consists have been discussed and it becomes clear that although crucial for a healthy life, overfeeding on either of these macronutrients can have pathological outcomes potentially shortening lifespan. This paper focussed on macronutrients in CR. The effects of micronutrients e.g. vitamins on life-extension and health have shown potential but are beyond the scope of this paper (79, 80).

No lifelong longitudinal studies on CR in humans have been done, for obvious reasons. However, the CALERIE-study did report the potentials of CR in humans. Cardiometabolic risk factors including LDL- and HDL cholesterol, insulin, and blood pressure all improved upon 25% CR with no negative psychological or cognitive side-effects. The CALERIE- study did not take into account the sexual dimorphisms that also exist in humans. Women naturally have higher body fat. Also, women typically carry less body weight than men meaning a lower basal metabolic rate. The effects hormonal changes that occur around menstruation and the potential loss of menstruation upon CR are also not yet understood.

CR has shown potentials on improving health span in most organisms and even life-extension in some. The exact mechanisms in which CR works are still being investigated. Future studies on CR should discriminate for sex- differences, both in model-organisms as for human studies. Furthermore, the roles of each of the macronutrients and the pathways in which they interplay are far from fully understood and understanding than could help with developing a proper diet-formulation. Ideally this diet- formulation would avoid the sensation of hunger to occur, making it easier for the broad public to apply the diet in their life-style. CR-mimetics are a hypothetical class of dietary supplements or drug candidates that would, in principle, mimic the substantial anti-aging effects that calorie restriction (CR) (81). This field of research will gain interest in the coming years since it is the sustainable method to apply CR on humans.

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