SEX DIMORPHIC EFFECTS IN CALORIE RESTRICTION AND CR MIMETICS ON LONGEVITY

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Abstract

The ageing population is an increasing problem worldwide. Calorie restriction (CR) is seen as a promising intervention that prevents age-associated diseases and extends longevity in most animal models studied so far. CR is defined as the reduction of energy intake without malnutrition. And obstacle is that the precise calorie intake for the optimal outcome is hard to determine, as this is influenced by a lot of factors. With therapeutic intervention it is possible to activate the same pathways, but without the adverse effects. There have been multiple reports of sex-dimorphic effects in response to CR and CR mimetics. More research is needed to identify these effects to utilize CR to its full potential.

In this report the sex-dimorphic effects on calorie restriction as a longevity intervention will be explored. In the main findings this will be done by discussing the nutrient sensing pathways known to be affected by CR. There are some pathways that not only play a role in nutrient sensing, but also adjust cellular processes. After this the connections between the pathways and their regulation by CR are explained. The connections between the pathways are proven to be important in the response to CR. Finally, the drug based longevity interventions and their sex-dimorphism are discussed. It has been concluded that there are a lot of reports about sex-dimorphism in response to CR and CR mimetics, but there is a great need for more extensive research on the subject.

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Introduction

The ageing population is an increasing problem worldwide. According to the World Health Organisation(WHO) the number of people aged 60 years or older will rise from 900 million to 2 billion between 2015 and 2050. That is moving from 12% to 22% of the total global population.[1] The transition to an aged society leads to some serious financial challenges, including retirement and health care costs. As there are more medical possibilities the dilemma grows: to what extent should public money be spent on expensive medical care to save these few, mostly elderly, people? Another problem that arises is long-term care, with the differential gender mortalities. Not only is there an ageing population, it is also mostly female.[2] On average the lifespan of people in western countries is 80 years, but there are a lot of people who are only healthy till the age of 50. So there is a gap between the health and life span during which these elderly people are not able to fully participate in society. There is a need for interventions to close this gap.[3]

Calorie restriction (CR) is a non-genetic intervention that prevents age-associated diseases and extends longevity in most animal models studied so far. CR is defined as the reduction of energy intake without malnutrition, with a reduction of ≥10% in human studies and usually 20% or higher in rodent species.[4] About 30% of the animals on CR die at an old age without age-related diseases. Thus in these animals health span is equal to lifespan.[3]
CR intervention in humans is studied in the CALERIE trial(Comprehensive Assessment of Long term Effects of Reducing Intake of Energy). This study provides evidence that sustained CR is beneficial for the life span and without negative effects on the quality of life in nonobese humans. It achieved a degree of CR sufficient to affect some, but not all, pathways found in animal studies that induce longevity. More research is needed for the full potential impact of CR on human life and health span.[5]

It is hard to determine the precise calorie intake needed for optimal health, as this is influenced by a lot of factors like genetic background, energy expenditure etc. and therefore varies for each individual. In some populations any amount of CR could even be harmful, for example in individuals with limited body fat stores (a BMI of 18.5). Most people find it difficult to stick to such a strict diet. Insight in the mechanisms responsible for the increase in health span in response to CR may lead to potential targets for therapeutic intervention. With therapeutic intervention you could activate the same pathways, but without the adverse effects. Therefore, additional research is needed to identify the mechanisms responsible.[6]

Another factor involved in the precise calorie intake needed for optimal health is sex. There is more research needed to explore the sex-dimorphic effects, as most existing studies have favoured the use of male samples. There is a need for inclusion of sex as a biological variable, instead of treating it like a confounding factor.[7] When treating sex as a biological variable, differences appear in the effectiveness of CR as an longevity intervention. Research in various animal models found differences in the sexes with CR. Research in Drosophila melanogaster reported a greater extension of lifespan in females vs. males with CR.[8] A greater effect of CR on females has also been shown in mice.[9] There have also been reports of sex-dimorphic effects in drug-based longevity responses, like drugs that target signalling pathways downstream of CR.[10]

In this report the sex-dimorphic effects on calorie restriction as a longevity intervention will be explored. In the main findings this will be done by discussing the nutrient sensing pathways known to be affected by CR, the underlying mechanisms of CR and the sex-dimorphic effects on CR. In addition the drug based longevity interventions and their sex-dimorphism will be explained.

Main findings

The nutrient sensing pathways

There are some pathways that play a role in nutrient sensing, and adjust cellular processes like gene expression or protein activity to the nutrient supply. These pathways also act as regulators in ageing. The pathways that will be discussed here are the TOR(target-of-rapamycin), AMPK(AMP-activated protein kinase), IIS (Insulin/insulin-like growth factor-1(IGF-1)) and the sirtuin pathway.

mTOR pathway

The serine/threonine kinase mTOR (mammalian TOR) is the catalytic subunit of two specific complexes, mTORC1 and mTORC2, which differ in their protein build-up. They also differ in their downstream signalling effects and sensitivity to the drug rapamycin.[11] Rapamycin, an anticancer and immunosuppressive drug, inhibits mTOR. While rapamycin acutely and directly inhibits mTORC1, only chronic administration of rapamycin can inhibit mTORC2 in some, but not all, cell lines or tissues.[12] mTORC1 integrates signals from intra- and extracellular cues, such as: amino acids, growth factors, energy, and more. mTORC1 is involved in protein synthesis through the direct phosphorylation of two targets: p70S6 kinase(S6K1) and members of eIF4E, binding protein family(4E-BPs). Through phosphorylation mTORC1 inhibits 4E-BPs, by promoting their dissociation from the translational initiation factor eIF4E, thereby allowing 5’ cap-dependent mRNA translation. Through phosphorylation of S6K1 mTORC1 promotes translation of a number of mRNAs, that contain TOP motifs. These mRNAs mostly code for ribosomal proteins and regulators of translation which together boosts general translation.[13]

Furthermore, mTORC1 controls autophagy by direct phosphorylation of its key components, ULK1, or by modulation of the localization of the transcription factor TFEB.[14] During nutrient deficiency mTORC1 is inhibited, causing ULK1 to be rapidly dephosphorylated, which leads to the activation of ULK1 kinase and induction of autophagy. In mammals, mTORC1 regulates autophagy under nutrient-rich conditions through directly phosphorylating and suppressing the ULK1 complex.[15]

In addition, mTORC1 regulates lipid synthesis, glucose metabolism and mitochondrial function through phosphorylation of different transcription factors like SREBP and PGC1α. Thus, mTOR integrates information from upstream nutrient cues, like signals from IIS and AMPK, to regulate metabolic processes.[11]
mTOR has influence on longevity, as loss of function of the mTOR serine/threonine kinase extends lifespan in *C. elegans*[16], *Drosophila* and mice.[17] A study in mice interestingly found that genetic inhibition of mTORC2 reduces male lifespan but does not affect females.[18]

AMPK pathway

AMPK is a complex of a catalytic subunit and two regulatory subunits, its kinase activity is activated or increased by direct AMP binding and by upstream regulatory kinases responding to cellular AMP levels. Thus, AMPK is regulated by the AMP/ATP ratio. AMPK activity stimulates energy production, for example by promotion of glucose uptake and fatty acid oxidation, in response to low energy levels. In addition a lot of metabolic processes are influenced by AMPK by its phosphorylation of enzymes, regulatory proteins and other cellular components. Examples of metabolic processes influenced are protein, glycogen and fatty acid synthesis, and glucose uptake.[19]

AMPK also down-regulates pathways involved in biosynthesis of lipids, carbohydrates, proteins or ribosomal RNA to reduce cellular energy consumption.[11] AMPK also influences longevity, as it phosphorylates ULK1 and thereby promotes autophagy. Activated AMPK also phosphorylates FoxO(fork head box O) transcription factors, which results in their increased transcriptional activity. FoxO transcription factors promote cellular processes that support energy production or conservation, maintenance of reproductive function, and lifespan extension.[11] AMPK activation increases lifespan in C. elegans.[20]

IIS (Insulin/insulin-like growth factor-1(IGF-1)) signalling pathway

The IIS pathway acts through the PI3-kinase(PI13K)/AKT kinase signalling cascade, and is initiated by insulin or insulin-like peptides and IGF-1. These peptides are secreted in response to food or the sensory perception of food, and bind to insulin/IGF tyrosine kinase receptors. The receptors are composed of α and β subunits. The activated receptor transduces the signal to the phosphatidylinositol 3-kinase (PI-3K) which in turn converts phosphatidylinositol 4,5-bisphosphate (PIP2) into phosphatidylinositol 3,4,5-bisphosphate (PIP3). Increased levels of PIP3 activate the protein kinase AKT, which phosphorylates the FoxO transcription factors and promotes their retention in the cytoplasm thereby preventing their function. FoxO proteins act as a molecular switch for cells to adapt and show metabolic stability under conditions of nutrient shortage. The proteins respond to both nutritional and stress signals. FoxO transcription factors also increase insulin sensitivity by inciting the expression of insulin receptor and substrate.[21]

IGF-I induces stimulation of cell proliferation and growth, inhibition of apoptosis and it mimics some of the insulin effects.[22] Nutrient abundance leads to increased IGF-1 and mTORC1 signalling, which promote cellular processes that support energy storage or consumption, increased reproduction, and growth.[11]
GH(growth hormone) stimulates IGF production and release. IIS influences longevity, as altering genes involved in the IIS pathway and GH causes longevity in mice.[22] In humans insulin insensitivity decreases with age. Individuals who reach an old age tend to have low IGF-1 levels.[23]
Genetic variation within the FoxO3A gene is strongly associated with human longevity. Long-lived men exhibited several biological markers for greater insulin sensitivity, which was associated with homozygosity for the FoxO3A GG genotype.[24]

The sirtuin pathway

Sirtuins regulate activation, or deactivation, of their protein targets by deacetylation or ADP-ribosyl transferase activity. In humans seven sirtuin homologues have been discovered, which differ in their cellular region and functions. Three sirtuin homologues (SIRT3, SIRT4 and SIRT5) regulate mitochondrial functions. The best researched homologue, SIRT1 (sirtuin 1), is mainly located in the nucleus. SIRT1 regulates transcriptional regulation, genomic silencing and other epigenetic factors. SIRT1 is also located in the cytosol, where it regulates metabolism and nutrient sensing. SIRT1 consists of an activation site, a NAD+-binding site, and a zinc-binding site. Sirtuins are very important in the regulation of metabolism as their function depends on NAD+ levels. NAD+ is a regulator of metabolism, because of its influence on glycolysis, the TCA (tricarboxylic acid) cycle and the electron transport chain.[25]

When nutrient levels are low, like during fasting, NAD+ levels are higher. SIRT1 influences the switch to gluconeogenesis and glucose production in the liver. It also stimulates mitochondrial biogenesis and fatty acid oxidation.[26] It is demonstrated that sirtuins play an important role in ageing and age-related diseases through regulation of multiple cellular processes. SIRT1 deacetylates and activates FoxO transcription factors, it activates autophagy by deacetylating autophagy regulators.[27] In yeast life span is modulated by the SIR genes. Overexpression of Sir2p promotes longevity in yeast.[28] Overexpression of SIRT6 extends lifespan in male mice. These male mice showed lower serum levels of IGF1, higher levels of IGF-binding protein 1 and altered phosphorylation levels of components in IGF1 signalling. [29] In humans, a variety of SNPs (single nucleotide polymorphisms) associated with sirtuin genes have been identified to correlate with healthy ageing and longevity.[30] For example SNPs linked to the SIRT1 gene have been found in long-lived populations of Chinese people.[31]

Connections between pathways and their regulation by CR

To understand how CR works as intervention to promote longevity the main molecular mechanisms will be explained. The decrease in calories activates pathways and systems involved in a more efficient metabolism, a higher protection against damage, activation of remodelling mechanisms and shuts down pathways that require a lot of energy, for example proliferation. In all organisms studied, these pathways regulate each other. These complex interactions are visualized in figure 1.[32] The most important effects of CR are modulation of mitochondrial activity and a decrease in oxidative damage. Modulation of mitochondrial activity occurs by inhibition of IGF-1 and TOR, and by activation of AMPK and sirtuins. Reduction of oxidative damage occurs by induction of endogenous antioxidant systems, at least partially mediated by FoxO transcription factors.
The relationship between calorie intake and longevity is a U-shaped curve. Both abundance of nutrients and malnutrition negatively impact survival.[33] It has also been proven that the longer the duration of CR, the greater the positive effect on longevity.[27]

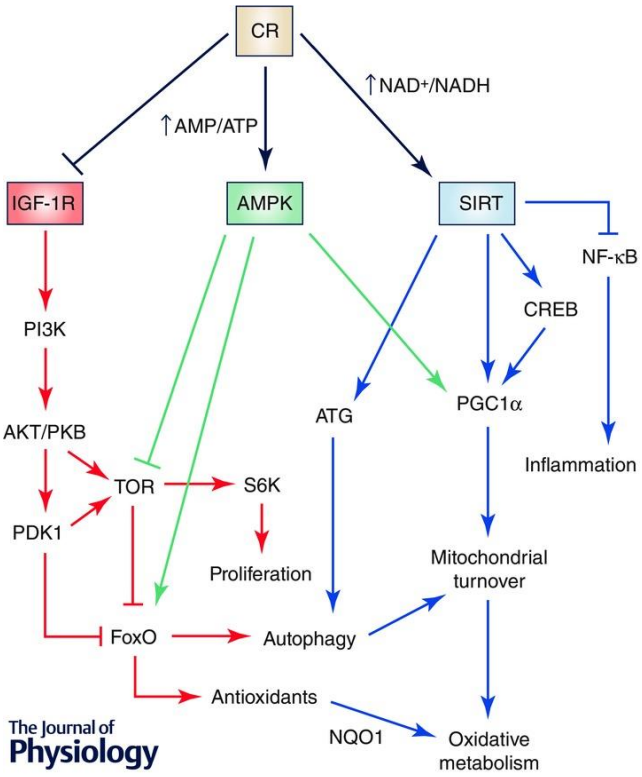

Figure 1 The complex interactions between the molecular pathways in CR[32]

The primary pathways involved in CR are the mTOR and IGF-1 pathway. CR reduces the levels of insulin, IGF-1, glucose, amino acids, lipids and other nutrients in humans and other vertebrates. CR thereby inhibits signalling of these pathways.[34] CR inhibits the IGF-1 cascade and thereby prevents protein synthesis by inhibiting TOR and S6K. This results in activation of FoxO transcription factors, which are seen as important downstream mediators of effects on health- and lifespan of the IIS pathway. CR downregulates mTOR via downregulation of the IIS pathway and through reduced amino acid availability, and a decrease in TOR activity itself is associated with longevity. A decrease in TOR leads to a decrease in S6K activity, and the genetic knockout of the S6K1 gene extends lifespan and delays the progression of age-related diseases in female mice.[35]

AMPK is activated by an increase in the AMP/ATP ratio, this happens when cells are deprived of glucose like in CR. In several organisms AMPK is associated with longevity. In Drosophila it has been demonstrated that an increase in AMPK activity is associated with a longer lifespan, while its inhibition shortens it.[36] But there have also been reports that its activity is not affected by CR, in mice CR did not alter AMPK activity.[37]

The AMPK and TOR pathways are linked together, as opposing signalling pathways involved in the sensing of availability of nutrients. AMPK is switched on by lack of nutrients and inhibits cell growth, while TOR is switched on by nutrient availability and promotes cell growth.[38] AMPK switches off the mTORC1 complex by two mechanisms. AMPK phosphorylates TSC2(tuberous Sclerosis Complex 2) at Thr1271 and Ser1387. TSC functions as an upstream mTORC1 inhibitor.[39] AMPK also directly phosphorylates the RAPTOR (regulatory associated protein) component of mTORC1 at two sites, Ser722 and Ser792.[38] RAPTOR functions as a scaffolding protein that facilitates the recruitment of substrates to the mTOR kinase.[40]

The IIS and mTOR pathway are directly connected as AKT phosphorylates TSC2 and inhibits the TSC complex, which activates mTORC1. S6K1 phosphorylates IRS1(insulin receptor substrate 1), thereby creating a negative feedback loop inhibiting insulin receptor signalling.[41]

Sirtuins act as nutrient sensors by responding to the NAD+/NADH ratio. When nutrients decrease, like in CR, NAD+ accumulate and sirtuins are activated.[32] SIRT1 has been proven to enhance insulin sensitivity in mice during CR.[42] SIRT1 and 6 are also important down regulators of IGF-1 and its target mTOR.[43]

Sex dimorphism in calorie restriction

There have been several reports of sex dimorphism in the effects of calorie restriction in model organisms. It is important to examine these differences and underlying mechanisms to be able to develop drugs to prolong human health span, of both men and women.

Mice

Multiple studies have reported positive effects on longevity with CR. CR increases insulin sensitivity and attenuates β-amyloid deposition in an Alzheimer model.[3] A study in mice found sex-dimorphic effects in different severities of CR, the researchers used two strains of female and male C57BL/6J and DBA/2J mice (referred to as B6 and D2). In the first strain, B6, the researchers report improved survival with 20% CR, with a mean lifespan extension of 40,6% in females and 24,4% in males compared to ad libitum controls. However, in this strain 40% CR did not show a beneficial response in female survival. B6 males did show beneficial effects with 40% CR, but not as much as with 20% CR.

In the second strain, D2, the researchers report 37,3% and 29,8% increases in maximum lifespan in both sexes with 40% CR. However, the D2 males on 40% did show a 18,8% reduction in first quarter lifespan. This difference between sexes and strains could be due to a difference in body composition. The researchers suggest that the preservation of fat mass in CR mice plays a protective role in survival and lifespan extension.[9]

Ambush feeding Copepod

Copepods are small crustaceans and earlier studies found sex-specific lifespan differences. Calorie restriction of the marine cyclopoid copepod *Oithona davisae* lowered mortality rates and increased survival. The males of this species generally have a shorter life span, this may be due to the fact that they are active swimmers when searching for females.[44]

In the experiment they observed different effects of CR on the lifespan. In female copepods CR reduced age-specific mortality rates and extended the life expectancy, in comparison with copepods under abundant foods. Female copepods under CR also had a longer reproductive period. They did not observe significant effects of CR on the longevity of male copepods. The researchers suggest that this is due to the higher rates of energy expenditure and consequent oxidative damage in males. [45]

Caenorhabditis elegans

Another study tried to identify the sex dimorphic effects of CR in the nematode *Caenorhabditis elegans*. C. elegans has two sexes, male and hermaphrodite. The males can shorten the lifespan of hermaphrodites, even without mating.[46] In addition, successful fertilization and subsequent proliferation of the germline shorten the lifespan of hermaphrodites. These effects of the sexual environment on lifespan are mediated by nutrient-sensing pathways. This suggests a link between CR and sex. Thus, the modulation of these pathways has different effects on the lifespan of the sexes. [47]

The study reports an extreme sexual dimorphism in the effects of CR. The hermaphrodites show longevity responses to CR, but the males show little response and maintain reproductive ability. The researchers suggest that this may be due to the regulation of the sex determination pathway and the steroid hormone receptor DAF-12 on the CR responsiveness. The results show that DAF-12 is not required for CR-induced longevity, but it is required for CR unresponsiveness in longevity in males.[47]

Drosophila melanogaster

In a study of the fruit fly, *Drosophila melanogaster*, researchers tested for sex differences in different severities of CR. They report that the response of life span and mortality rates to CR differs between female and male *Drosophila,* but they both show a positive response. Females were more responsive to CR and their life span peaked at a higher food concentration. Male lifespan peaks at a lower food concentration and was less sensitive to CR. The researchers give two possible explanations for the observed sex bias: there are differences in nutrient demand and energy distribution between the sexes, or there might be differences in IIS signalling and its response to CR between the sexes.[8]

Humans

The CALERIE study found that weight loss with CR is significantly affected by sex, men in the CR group lost more weight than women. However, the percentage of weight loss was not significantly different between the sexes. Body composition changed significantly over the 2 years, with relatively higher FFM (fat free mass) in both sexes.[48] Ageing is associated with a decrease in the quantity and quality of FFM, which affects the physical function and increases the risk of metabolic disorders.[49]

The sex-dimorphic effects of CR found in this could be due to the fact that there were less male subjects enrolled in the study. The effects of CR on life span are not known, this could be the same or different between the sexes.[48]

Drug based pro-longevity interventions and their sex-dimorphic effects

Multiple drugs have been proven to have a similar effect as CR on longevity in model organisms. These mimetics work via the same nutrient sensing pathways as affected by CR. Examples of downstream mimetics are rapamycin (an inhibitor of mTOR), metformin (an activator of AMPK), resveratrol (an activator of AMPK and SIRT1), and spermidine (inducer of autophagy). An example of an upstream CR mimetic is acarbose (glycosidase inhibitor).

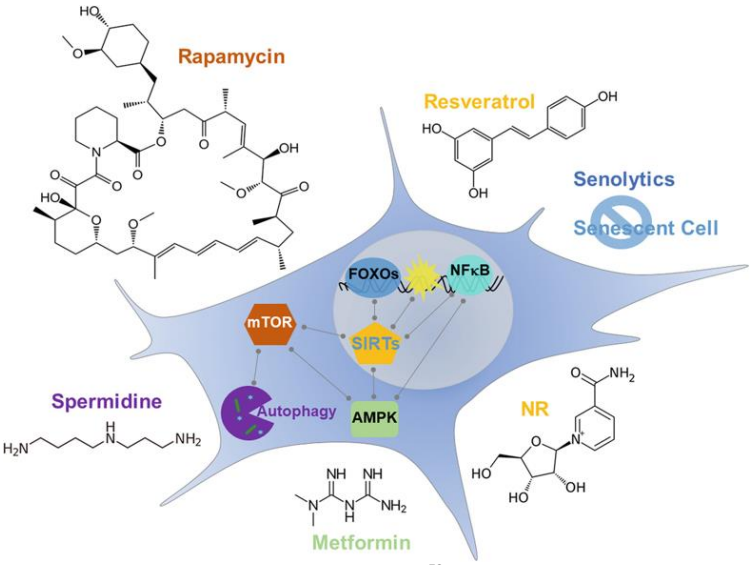

Figure 2 CR mimetics and their structures[50]

There have also been reports of sex-dimorphism in drug based pro-longevity interventions. The most researched drug with sex-dimorphic effects is rapamycin. Others are metformin, resveratrol and acarbose. There have not been reports of sex-dimorphic effects on Spermidine.

Rapamycin

Rapamycin is an immunosuppressive drug, that has been used to prevent transplant rejection and treat autoimmune diseases.[51] It associates with the intracellular protein FKBP12 and inhibits mTORC1 activity by blocking the interaction between mTOR and RAPTOR. Acute treatment with rapamycin inhibits mTORC1 signalling, and thereby restricts growth and promotes longevity without reducing insulin sensitivity. Chronic treatment with rapamycin inhibits both mTORC1 and mTORC2, restricting growth and impairing insulin signalling, which potentially also contributes to longevity.[52]

Rapamycin has been proven to promote longevity in yeast, worms, flies and mice.[52] However rapamycin also generates serious side effects, because it is an immunosuppressive drug. This is why it may not be a good long-term anti-ageing drug for humans.[51]

Rapamycin leads to a larger percentage increase of longevity in female mice than in male mice. The data in this study showed higher levels of rapamycin in the blood of female mice compared to that of males at varying doses. This difference may be due to: sex-hormones, sex-dimorphism in immune tone or sex-specific differences in fat-distribution.[53] Another study in mice also found higher blood concentrations of rapamycin in females. The study suggests that this could be due to greater bioavailability of rapamycin in females.[10]

A study with a very high dose of rapamycin for a short time reports prolonged life span in male mice. In the female mice the researchers found serious side effects, a shift towards aggressive hematopoietic cancers was observed. In males no side effects were found with this dosage.[54]

Metformin

Metformin is an anti-diabetic drug which is able to suppress hepatic gluconeogenesis, enhance peripheral glucose uptake and decrease absorption of glucose from the gastrointestinal tract. It works by activation of AMPK to inhibit expression of hepatic gluconeogenic genes and to stimulate GLUT4 translocation to the plasma membrane to improve insulin-independent glucose uptake. Metformin also inhibits mTORC1 independently of AMPK.[50]

Metformin has been proven to prolong health and life span in C. elegans. This study showed that metformin increases the lifespan via a mechanism independent of the IIS pathway but with the same features as CR. The researchers suggest that lifespan extension is executed via mechanisms that are conserved from nematodes to humans.[55] Metformin has been shown to lengthen the lifespan and attenuate the detrimental effects of ageing in male mice.[56] In humans the MILES (metformin in longevity study) was performed, which showed that 6 weeks of metformin can improve age-associated metabolic derangements in glucose intolerant older adults. But more research is needed to explain metformin's mechanisms in humans.[57]

There has been a lot of research about the sex-dimorphic effects of metformin treatment in mice. Anisimov et al. did a study in two parts on this subject: The first study gave the mice metformin from the age of 3 months. It reports an increase of median life span in females, and a decrease in median lifespan in males. Maximum lifespan was increased in both.[58]
In the second study metformin was given to the mice just days after birth. It reports the opposite: female lifespan decreased and male life span increased. Possible explanations for this are: the mechanism of ageing is different in males and females, or the early treatment reprogrammed the hypothalamic control of energy homeostasis in males and females differently.[59]

Resveratrol

Resveratrol is a kind of polyphenol, and has anti-cancer properties. It induces longevity by reducing mitochondrial dysfunction, oxidative damage, and chronic inflammation and by elevating insulin sensitivity. Resveratrol also improves vascular function and activates longevity genes like sirtuins. It activates SIRT1 and SIRT2 and it represses NF-kB.[60]

Resveratrol increases longevity in various species, its ability has been proven from yeast to mammals. In honey bees resveratrol prolonged their life span up to 38%.[61] However, there have been conflicting reports on resveratrol in other species. In Drosophila there have been reports of successful lifespan extension, but also reports with no significant effects.[62] Resveratrol has only been proven to improve longevity in mice on a high calorie diet.[63] Resveratrol did not increase survival in mice fed a standard diet.[64] In humans long-term treatment of resveratrol improves cognitive abilities in elderly patients. This study showed that 26 weeks of resveratrol improved memory performance in elderly people, they also observed a significant reduction of body fat.[60]

Resveratrol is classified as a phytoestrogen(plant-derived) as it can bind to the oestrogen receptor and modulate oestrogen receptor signalling. Because of this researchers suggest that the effects of resveratrol could be sex-dependent. Louis et al. researched the cardioprotective effects of resveratrol, which are more present in females. They describe that several points in the oestrogen signalling pathway may be regulated by resveratrol. But the sex-dimorphic effects they found could also be due to the fact that the pathology, cardiovascular disease, is sex-dependent. They suggest that premenopausal women have a more favourable outcome with resveratrol than males. The experiment the researchers did in rats confirms this finding.[65]

Spermidine

Spermidine is a polyamine, it induces autophagy in multiple model organisms. It works by inhibiting the activity of several acetyltransferases that acetylate and thereby inhibit components of the autophagy machinery. Spermidine also inhibits mTORC1 and activates AMPK, which further stimulates autophagy.[66] It suppresses tumorigenesis, enhances anticancer immune response, stimulates memory T cell formation, and mediates neuroprotection.[67]

Spermidine promotes longevity in yeast, nematodes and flies. However, the mechanism of its anti-ageing effect is not fully clear, as it is not sure whether the longevity is due to the deacetylation of histones or non-histone proteins. [68] In humans, the spermidine concentration declines during ageing in different organs which possibly contributes to the ageing process.[69]

So far there are no reports of sex-dimorphic effects on spermidine. More research is needed to find possible effects.

Acarbose

Acarbose is an α-glycosidase inhibitor, which is used to treat diabetes worldwide. It is an oligosaccharide that works as a competitive inhibitor to carbohydrate breakdown in the small intestine, because it has a greater affinity than sucrose for α-glucosidase. It results in reduced enzymatic degradation and absorption of glucose from complex carbohydrates.[70]

In rats, chronic acarbose treatment reduces body weight and body fat. It also improves glucose dysregulation associated with ageing.[71] In mice acarbose leads to prolonged longevity.[72]

Weight reductions due to acarbose are greater in female mice than in males, but the median lifespan increased more in males than females. In males it increased by 22% and in females by only 5%. Acarbose reduced fasting insulin significantly in males but not in females. Because of these findings the researchers suggest that the sex-dimorphic effects are not due to the changes in body weight but rather to a change in insulin sensitivity.[72]
Another study in mice found that acarbose also leads to male-specific improvements in glucose tolerance and higher hepatic mTORC2 activity. These researchers suggest that the sex-dimorphic effects are caused by gonadal hormones as castrated males do not show improvements with acarbose.[73]

Conclusion

The world population is ageing, which leads to serious financial challenges. These challenges are caused by the fact that there is a gap between the health span and life span in these elderly people, in which they cannot fully participate in society. A potential solution is calorie restriction, an intervention that extends health span and life span in most animal models so far. It is, however, hard to determine the optimal calorie intake, as this varies for each individual. An example of a factor involved is sex, but there has not been a lot of research into this as it is mostly treated as a confounding factor. The goal of this thesis was to explore the sex-dimorphic effects on calorie restriction as a longevity intervention.

There are different nutrient sensing pathways which are not only important for energy homeostasis, but also in different cellular processes that are also involved in cellular and organismal ageing. An important nutrient sensing pathway is the mTOR pathway, which integrates information from the IIS and AMPK pathways to regulate metabolic processes. CR downregulates mTOR, and a decrease in TOR activity is associated with longevity. Sex-dimorphism in CR response has been described in multiple model organisms, but not in humans. The CALERIE study did find sex-dimorphism in the effects of CR, but this could be caused by the fact that there were less males than females enrolled in the study. Most people find it hard to stick to such a strict diet, which is why drug based pro-longevity interventions using CR mimetics are under development. These drugs have been proven to have a similar effect as CR using animal models. Multiple studies report sex-dependent effects of these mimetics, with alternating preference in males or females.

Because of these strong sex biased effects of most of the already tested CR mimetics there is an urgent need for extensive research on the subject. While proof of these effects has been found, their underlying mechanisms are still mysterious. In further research it would be important to test out different concentrations and different time windows for the treatment, in both males and females. It could also be interesting to look at the timing of the treatment, as some studies pointed out that the time in the life of the organism also has influence. In later studies it is also important to look at the side effects, as these could also differ between sexes and time windows. Some mimetics could even have harmful side effects in one sex but not in the other. With insight into these mechanisms you could possibly find the best intervention per sex, and maybe even per individual based on sex, age and organism.

It would also be interesting to explore the possible explanations named in this report, to see whether the sex determination pathways or hormones discussed earlier also have influence in other organisms. Future studies will also help to answer questions about how ageing works and its mysterious underlying mechanisms. It would be interesting to see how species-specific lifespans are determined.

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