The insulin/IGF-1, the sirtuin and the mTOR pathway.

How alterations of these pathways could affect age-related diseases and lifespan.

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ABSTRACT

The sirtuin pathway, the insulin/insulin-like growth factor-1 signalling pathway and the mTOR pathway have been found to regulate lifespan in various lower organisms. However the mechanism by which these pathways affect lifespan in mice is far less well defined. Alterations to each of these pathways have effects on aging and age-dependent diseases, such as diabetes, obesity, heart failure and cancer. The effects on lifespan, however, are more variable. While some studies show impressive extension of lifespan by alteration of these pathways, other studies find milder extensions or even no extension at all. Overexpression of most sirtuins do not extent lifespan, only overexpression of Sirt6 might improve longevity. The growth hormone/IGF-1 signalling pathway does affect lifespan, but these alterations can exhibit paradoxical results. The mTOR pathway effects lifespan positively, however most results are sex-specific. Even though, lifespan prolonging effects are difficult to demonstrate in mouse models, because of interaction of multiple pathways, the use of these models does provide insights into the mechanisms of metabolic pathways and points out potential drug targets, for the treatment of age-dependent diseases in humans. This is because mice are more similar to humans than lower organisms, such as C. elegans, are. Taken together alterations of metabolic pathways can improve health span in mice, but to state that they definitely improve lifespan in mice certainly needs further investigation.

Index

Abbreviation list

Akt	Protein kinase B
AMPK	AMP-activated protein kinase
BubR	Budding uninhibited by benzimidazole-related
CR	Calorie restriction
CVD	Cardiovascular disease
EGFR	Epidermal growth factor receptor
FGF	Fibroblast growth factor
FKBP	12-kDa FK506-binding protein
Foxo	Forkhead box O
GH	Growth hormone
GHRH	Growth hormone releasing hormone
GSK	Glycose synthase kinase
IGF-1	Insulin-like growth factor 1
IGF1R	Insulin-like growth factor 1 Receptor
IIS	Insulin/IGF-s signalling
KO	Knockout
mTOR	Mammalian target of rapamycin
mTORC	mTOR complex
NAD^+	Nicotinamide adenine dinucleotide
NFAT	Nuclear factor of activated T-cells
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PGC	Peroxisome proliferator-activated receptor-gamma coactivator
PI3K	Phosphoinositide 3-kinase
Rheb	Ras homolog enriched in brain
Sirt	Sirtuin
S6K	Ribosomal protein S6 kinase
TBC1D7	Tre2-Bub2-Cdc16 TBC 1 domain family, member 7
TGF	Tumour growth factor
TSC	Tuberous sclerosis complex
TSG	Tetrahydroxystilbene glucoside
T2D	Type 2 Diabetes
WT	Wildtype
4E-BP	Eukaryotic translation initiation factor 4E-binding protein

Introduction

One of the biggest risk factors of developing a variety of diseases is aging, a process that can be described in many different ways. It can be described as a process that is accompanied by functional decline and increased risk of developing age-related diseases[1]. In the sense of individual organisms aging is the process of getting older that is genetically determined and can be changed by environmental conditions. In a more specific sense it refers to the halt to the dividing of single cell, which is also known as senescence. Aging could also be described as the cellular accumulation of DNA damage until cellular systems seize to work. Besides all the different definitions of aging, dysregulation of homeostatic mechanisms is a main characteristic this process. This dysregulation can be clearly seen in metabolism. Insulin resistance, decreased levels of growth hormone (GH) and decreased levels of insulin-like growth factor-1 are prominent hallmarks of aging. These metabolic changes, are main contributors to different metabolic diseases, such as type II Diabetes (T2D), cardiovascular disease (CVD) and stroke[2]. Preventing or delaying the onset of these metabolic changes during aging could prevent or delay development of these diseases and therefore could increase the health span. Moreover, preventing or delaying the onset of these metabolic changes could even increase lifespan.

Aging is a complex and intriguing process, because sooner or later, it will happen to all living organisms. For this reason, aging has been a frequently studied subject for the past few decades. Many studies have focused on ways the prolong lifespan or on diminishing the pathologies aging is accompanied with. Metabolic intermediates of metabolism have frequently been associated to aging. The other way around, many alterations of metabolic homeostasis have been found to prolong lifespan or at least health span. The best known lifespan prolonging intervention is caloric restriction (CR).. Across species, CR has been found to increase lifespan. However, how this process exactly works has yet to be uncovered completely[3]. Different metabolic signalling pathways have been found to mimic the effects of CR and therefore might be targeted to improve longevity. Herein, the most prominently investigated pathways in relation to aging, age-related diseases and prolonging lifespan will be discussed. These pathways are the sirtuin pathway[4], the insulin and insulin-like growth factor-1 pathway and the mTOR pathway. Normal functioning of these pathways as well as how these pathways change during aging will be discussed. However the focus of this assay will be on clarifying how alterations to these three pathways can extend lifespan.

The insulin/IGF-1 signalling pathway

As the name already suggests, insulin and insulin-like growth factor 1 (IGF-1) are key players in the insulin/IGF-1 signalling (IIS) pathway. Insulin and insulin-like proteins, are present in many different species such as mammals, fungi and Protista. Even unicellular eukaryotes contain insulin, suggesting molecular originating from an early common ancestor[5 6]. In modern day species, such as mice, primates and humans. Insulin is a peptide hormone secreted by β-cells of the pancreas. Secretion of insulin is stimulated by increased blood glucose levels. After secretion, insulin can bind the insulin receptor, through which insulin activity is mediated. This activity mainly regulates glucose uptake and inhibition of glucose production and secretion by the liver[7]. In hyperglycaemic conditions, however, cells lose sensitivity to insulin, which contributes to development of insulin resistance and type 2 Diabetes (T2D) as a consequence [8]. Besides glucose homeostasis, there is a wide range of physiological processes in which insulin plays a significant role. Insulin is involved in anabolic metabolism, such as fat storage[9] and protein synthesis[10]. On the other hand, low insulin levels stimulate catabolic metabolism[11], specifically that of body fat[12]. In this way, insulin is not only linked to diabetes, but also to overweight and obesity[13].

IGF-1 is similar to insulin when looking at molecular structure, however, they differ is function. IGF-1 is mainly involved in growth, development and anabolic processes, such as bone growth[14 15 16]. Although IGF-1, like insulin, can bind the insulin receptor, activity of IGF-1 is predominantly mediated by binding of the insulin-like growth factor-1 receptor (IGF1R). Production of IGF-1 takes place in endocrine tissues of the liver[17]. Secretion can be stimulated by growth hormone (GH) and inhibited by insulin and caloric restriction[18]. Upon receptor binding of IGF-1 or insulin, phosphoinositide 3-kinase (PI3K) becomes activated. Active PI3K, in turn, phosphorylates protein kinase B (Akt) and thereby activates it[19]. Akt has many different functions depending on the downstream targets it inhibits or stimulates. Via NF-κB activation, Akt regulates cell survival by inhibition of apoptosis [20 21]. By inhibition of glycogen synthase kinase 3 (GSK-3) Akt regulates glycogen synthesis[22]. Via phosphorylation and thereby inhibition of several Forkhead box O (Foxo) proteins Akt stimulates tumour development[23].

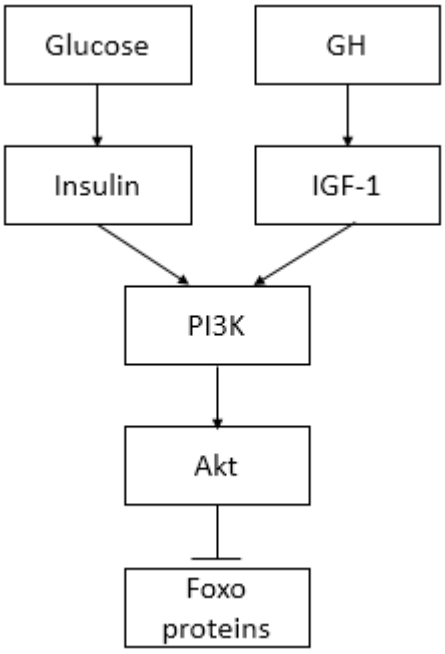

Figure 1. Schematic representation of the insulin/IGF-1 signalling pathway. Increases blood glucose levels stimulate insulin secretion. Growth hormone (GH) stimulates insulin-like growth factor 1 (IGF-1) secretion. Both insulin or IGF-1 binding to their receptor (insulin receptor and IGF-1 receptor resp.) phosphorylates and activates , phosphoinositide 3-kinase (PI3K), which in turn phosphorylates and activates protein kinase B (Akt). Akt phosphorylates several Foxo proteins leading to inhibition of activity of these Foxo proteins.

The IIS pathway during aging

During aging, activity of the IIS pathway changes, leading the various pathologies. For instance, insulin levels increase causing elevated risks of developing T2D and obesity. This increase in levels of insulin is mainly caused by insulin resistance in different tissues and the resulting upregulation of insulin secretion from the pancreas[24 25]. On the other hand, levels of IGF-1 peak during puberty, but decrease afterwards as part of the aging process in humans. In rodents however, the levels of serum IGF-1 remain high, even during aging[26]. In humans, declining levels of IGF-1 could be accounted to the decrease of GH levels and secretion during aging[27 28]. Decreased levels of GH result in impairment of IGF-1 secretion. Low levels of IGF-1, in turn, are associated with several conditions such as T2DM and CVD, including heart failure[29]. Contrastingly, age-dependent increased levels of IGF-1 are linked to some types of cancer[30 31].

The IIS-pathway and lifespan extension

Since changes to the IIS pathway are linked to aging and different age-associated diseases, altering the IIS pathway could counteract or prevent these changes during aging and, could potentially extend lifespan. Recent studies were able to demonstrate that insulin sensitivity is crucial for delaying the aging process. In mice with knockout (KO) of the growth hormone receptor (GHR) lower levels of insulin were found due to increased insulin sensitivity. These mice also showed decelerated senescence and more characteristics associated with slow-aging compared to WT mice[32]. This indicates that insulin sensitivity plays an essential role in the aging process. Besides slowing the aging process down, lower insulin levels and improved insulin sensitivity have been linked to increased lifespan. Male mice heterozygous for the Ins2g gene ($Ins2^{+/-}$), an ancestral insulin gene, age healthier compared to male mice homozygous for Ins2 ($Ins2^{+/+}$). $Ins2^{+/-}$ mice had lower levels of circulating insulin, which was associated with decreased glucose levels and increased insulin sensitivity. Most importantly, $Ins2^{+/-}$ mice showed significant lifespan extension compared to $Ins2^{+/+}$ mice[33]. This indicates that higher levels of insulin are involved in age-dependent IR and that lowering these insulin levels can extend lifespan.

However, lifespan extension is more likely to be due to increased insulin sensitivity and decreased glucose levels than due to decreased insulin levels, since high insulin levels are essentially not harmful. The impact of insulin levels are paradoxical since they depend on the insulin sensitivity of peripheral tissues. In diseases like T2D, insulin resistance and hyperglycaemia are common and have negative effects on lifespan. Hyperglycaemia is associated with many pathologies such as nephropathy[34], cardiomyopathy[35] and neuropathy[36]. In the case of insulin resistance low levels of insulin are not sufficient to take up glucose. This in turn causes inflammation and FGF23 upregulation, contributing to chronic kidney disease[37 38]. However, in tissues with high insulin sensitivity, lower levels insulin are rather healthy. All in all, increased insulin sensitivity seems to be critical in slowing the aging process down and prolonging lifespan. Pharmacologically, insulin sensitivity can be improved in mice by metformin, a drug used in T2D treatment. Metformin mimics the lifespan extending effects of CR, without reducing food intake and activates AMP-activated protein kinase (AMPK), which reduces oxidative damage and inflammation[39]. These effects of metformin treatment contribute to health and lifespan extension.

Like insulin, IGF-1 levels shows paradoxical effects of both high and low levels. In mice, impairing IGF-1 levels has been associated with lifespan extension[40], while other studies showed impaired health span after reducing serum IGF-1[41]. Since altering IGF-1 levels does not have clear effects on aging and lifespan, but IGF-1 signalling does seem to been involved in regulation of it, altering GH levels has been frequently studied. GH deficient mice are more long-lived than normal mice and IGF-1 heterozygous mice, which could be due to elevated mitochondrial metabolism and elevated adiponectin in the absence of GH[42]. GH-releasing hormone (GHRH) KO mice exhibit a deficiency in GH levels and also show extreme extension of lifespan[43]. This lifespan extension could be because of GH deficiency itself or because of impairment of targets more downstream of the GH/IGF-1 pathway.

Blocking IGF1R, for example, is one of these more downstream targets. IGF1R KO mice show a leaner phenotype and have intact glucose metabolism upon ageing compared to WT mice. However, IGF1R KO mice exhibit relatively high GH levels[44]. Furthermore, inhibiting the IGF1R in combination with the epidermal growth factor receptor (EGFR) enhances already existing treatments for cancer, such as radio chemotherapy. In colorectal cancer specifically, inhibition of both receptors in combination with radio chemotherapy shows more reduction of tumours than radio chemotherapy alone[45]. Beyond the beneficial effects of IGF1R KO on metabolism and tumour suppression, female mice heterozygous for IFG1R show significant increase of lifespan compared to WT mice[46]. Furthermore, body temperature during caloric restriction seems to play a role in lifespan extension. IGF-1 levels are decreased in response to CR[47], and result in lower body temperature and more efficient energy expenditure. Both pharmacological and genetic inhibition of the IGF1R can mimic these effects. This indicates that body temperature, CR and IGF-1 signalling, which all play a role in metabolism, aging, age-related diseases and lifespan, are all part of the same pathway[48]. Thus, both GH and IFG1R deficiency show capability of extending lifespan. This could be because of impaired GH/IGF-1 signalling as a result of both deficiencies. However, IFG1R deficiency-mediated lifespan extension was only found in female mice while GH deficiency-mediated lifespan extension was found in both sexes. This and the improved energy metabolism, indicates that GH deficiency has additional beneficial effects independent from reduced IGF-1 secretion.

Other downstream targets of the IIS pathway are specific subtypes of the Forkhead box O (FOXO) proteins. Especially Forkhead box O3 (FOXO3) seems to be involved in the aging process and longevity. FOXO3 is involved in the beneficial effects of caloric restriction (CR) on longevity. Under CR conditions, lifespan of Foxo3 heterozygous mice and of Foxo3 deficient mice does not significantly increase while in contrast, WT mice did show significant prolongation of lifespan, suggesting that Foxo3 is required for the lifespan extending effects of CR. When looking at tumour formation, CR led to decreased tumour prevalence in WT, Foxo3 heterozygous and Foxo3 deficient mice compared to mice fed ad libitum[49]. This indicates that Foxo3 is not required for the antineoplastic effects of CR. Foxo1, on the other hand, shows contrasting results with Foxo3. Foxo1 is involved in cancer suppression. Mice expressing Foxo1 heterozygously show an extension of lifespan to the same extent as WT mice after CR. However, WT showed more tumour suppression than Foxo1 heterozygous mice[50]. This indicates that Foxo1 is not required for lifespan extension, but is required for suppression of tumour development in response to CR.

The sirtuin pathway

Sirtuins are NAD^+-dependent deacetylases that play a prominent role in the regulation of various biological processes such as physiological homeostasis, metabolism and aging[51] [52] [53]. In complex mammals seven different types of sirtuins (SIRT1-7) have been found that are categorized according to amino acid sequence structure. Due to variation in their amino acid sequence, the different types of sirtuins have varying cellular localisation. SIRT1, SIRT6 and SIRT7 are predominately localized in the nucleus, while SIRT3, SIRT4 and SIRT5 are most prominently found in mitochondria. SIRT2 can mostly be found in cytoplasm, but has also been associated with nuclear proteins[54]. Due to different sub-cellular localizations, sirtuins exert varying cellular functions[55]. For the induction of sirtuin signalling, sirtuins are dependent of nicotinamide adenine dinucleotide (NAD^+). Since NAD^+ reflects energy levels, sirtuins can be seen as sensors of cellular energy conditions[56]. Increasing intracellular levels of NAD^+ upon low energy conditions leads to activation of sirtuins[57], while a decrease in NAD^+ levels, reflecting high energy conditions, leads to inhibition of sirtuins[58]. AMPK regulates this energy metabolism by increasing NAD^+ levels[59]. Furthermore, activity of the sirtuin pathway can be stimulated by a wide range of molecules, such as NAD precursors, long-chain fatty acids and plant phenols such as resveratrol[60] [61] [62]. Upon activation, sirtuins stimulate Foxo proteins and peroxisome proliferator-activated receptor-gamma coactivator (PCG) 1α which regulates mitochondrial biogenesis[63]. Furthermore, sirtuins inhibit NF-κB activation, which is involved in inflammation induction and cell survival[64].

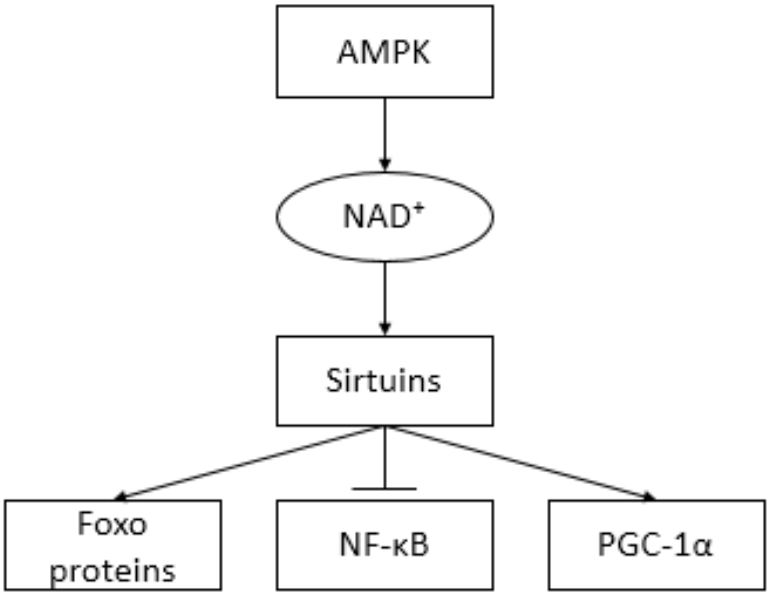

Figure 2. Schematic representation of the sirtuin pathway. AMP-activated protein kinase (AMPK) activates Sirt1 by increasing cellular levels of NAD^+. Activated Sirt1 stimulates the activity of several Foxo proteins and Peroxisome proliferator-activated receptor-gamma coactivator (PGC) 1α and inhibits the activity of NF-κB signalling.

The sirtuin pathway during aging

During aging, the levels of sirtuins decline, resulting in decreased activity of the pathway. Not only the levels decrease, activation of sirtuins is also impaired during aging. This can be attributed to the age-dependent decline of levels of NAD^+. This in turn, can be explained by the age-related increase of CD38 activity. CD38 is a NADase which is one of the most predominant enzymes in the degradation of NAD^+[65]. Thus, during aging, increased activity of CD38 results in more degradation of NAD^+, which lowers NAD^+ levels. In turn, this reduces the rate by which sirtuins become activated. Impaired sirtuin activity, together with decreased sirtuin levels are believed to contribute to various age-associated diseases, such as T2D[66 67], CVD[68 69] and various types of cancer[70]. The link between sirtuins and cancer, however, is far more complex. While sirtuin impairment is generally associated with tumorigenesis[71], also overexpression of sirtuins is found in tumours from different tissues[72 73].

When looking more specifically at the effects of decreased sirtuin activity per subtype, lowered activity of SIRT1 is linked to accumulation of damaged DNA[74], while SIRT3 inactivity is associated with fibrotic tissue formation as a result of hyperacetylation of glycose synthase kinase 3β (GSK3β). Hyperacetylation of GSK3β improves stability of transforming growth factor β (TGF-β), which in turn induces fibrotic tissue formation. On the other hand, increased levels of SIRT3 result in induced deacetylation and thereby activation of GSK3β, which inhibits cellular growth and tissue formation by blocking TGF-β signalling and synthesis[75]. Deficiency in the levels of SIRT6 induces hypoglycaemia and thereby increases the risk of developing DM or obesity[76]. Sir6 deficiency also results in progressed renal dysfunction due to increased inflammation and fibrotic tissue formation[77].

The sirtuin pathway and lifespan extension

The involvement of sirtuins in different age-related diseases indicates that altering sirtuin signalling could slow the development of these diseases down and thereby could potentially increase lifespan. Among the sirtuins, Sirt1 has been studied most intensively in terms of prolonging lifespan. Pharmacologically, Sirt1 can be activated by resveratrol[78], a natural phenol produced by plants of which over the past decade it has been claimed to extend lifespan. Indeed this has been demonstrated in short-lived vertebrates[79]. In mice resveratrol-induced activity of Sirt1 in osteoblast cells inhibited apoptosis induced by oxidative stress[80]. This effect is mediated by interaction of active Sirt1 with Foxo3. Foxo3 functions as a sensor of the insulin signalling pathway and is known to positively regulate lifespan. Sirt1 is able to deacetylate Foxo3 and therefore inhibits apoptotic pathways, but also to increases resistance of cells to oxidative stress[81].

Besides resistance to oxidative stress, resveratrol also enhances mitochondrial function and promotes anti-osteoporotic effects via mitofilin, an inner membrane protein of mitochondria induction[82]. All these effects of resveratrol contribute to extended health span, but resveratrol also contributes to extended lifespan. On a high calorie diet, mice treated with resveratrol live longer and show decreased IGF-1 levels, improved insulin sensitivity and increased AMPK and PGC-1α activity compared to mice on a high calorie diet without resveratrol supplementation[83]. This increased AMPK and PGC-1α activity indicates enhanced sirtuin signalling. Tetrahydroxystilbene glucoside (TSG) has been pointed out as another sirtuin signalling enhancer. TSG is a glucoside, which is one of the components of a Chinese anti-aging medicine and an analogue of resveratrol. TSG stimulates Sirt1 signalling and with that improves the physiological conditions of aged mice on a high caloric diet[84]. These results indicate that TSG has beneficial effects on aging. Thus, TSG could potentially be an interesting drug for the treatment of diseases associated with aging and high caloric consumption according to this research. However, TSG administration has only been demonstrated to delay aging symptoms, but has not been able to effectively and significantly prolong lifespan.

Other than Sirt1 activation, resveratrol also activates Sirt2. A recent study in rats showed that long-time treatment with resveratrol resulted in decreased levels of nitric oxide and lipoperoxidation in cardiac tissue, which are markers of oxidative stress and result in cardiac tissue ageing. Thus, resveratrol reduced age-related oxidative stress in cardiac muscle cells. However, no differences in activity of different enzymes of the antioxidant system were found, which indicates that resveratrol has direct protective effects on cardiac tissue instead of an indirect effect via activation of antioxidant enzymes[85]. Taken together, resveratrol enhances sirtuin signalling and is able to extend life span of mice on a high fat diet. TSG also enhances sirtuin signalling, but is only able to increase health span and not lifespan.

Sirt2 has been linked to cardiac aging more often. Recent research has demonstrated that Sirt2 is able to inhibit the development of cardiac hypertrophy, an important cause of heart failure. Cardiac hypertrophy is characterized by upregulation of nuclear factor of activated T-cells (NFAT) activity. Sirt2 deacetylates a specific isoform of NFAT, NFATc2, which diminishes nuclear localization of NFATc2 and thereby inhibits NFAT expression. This inhibition has beneficial effects on cardiac hypertrophy. Sirt2 deficient mice spontaneously develop hypertrophy in a pathological manner, while overexpression of Sirt2 in mice slows the process of hypertrophy development down, while[86]. Another study demonstrated that Sirt2 overexpression increases lifespan in mice hypomorphic for budding uninhibited by benzimidazole-related 1 (BubR1). BubR1 is a mitotic checkpoint kinase gene whose levels decline during the aging process, due to the age-dependent decline of NAD^+ and Sirt2 activity. In wildtype mice, overexpression of Sirt2 increased levels of BubR1, but only in BubR1 hypomorphic mice increase of lifespan had been observed[87]. The results of these studies taken together, Sirt2 overexpression slows down the aging process. However, lifespan extension has only been found in genetically modified mice and not in WT mice.

Sirt6 has more evidently been demonstrated to improve longevity in mice. Overexpression of Sirt6 in male mice results in lower levels of IGF-1, increased levels of IGF binding protein 1 and altered levels of phosphorylation of important proteins in the IGF-1 signalling pathway suggesting reduced signalling of this pathway or increased sensitivity, contributing to the extension of lifespan observed in this mouse model[88]. Furthermore, CR induced activity of Sirt6 shows inhibition of the aging process by suppression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signalling. NF-κB signalling is involved in inflammation, cellular senescence and organismal aging. Under CR conditions, Sirt6 expression increases which further contributes to the inhibition of NF- κB signalling[89]. In conclusion, Sirt6 seems to be able to delay the aging process in mice, however, lifespan extension due to Sirt6 has only been demonstrated in male mice, suggesting Sirt6-mediated lifespan regulation is sex-specific.

The mTOR pathway

The mammalian target of rapamycin (mTOR) pathway, more precisely called the PI3K/AKT/mTOR pathway regulates the cell cycle, cell growth and metabolism. The mTOR pathway is directly associated with cellular proliferation[90], cancer[91] and longevity[92]. Moreover, the mTOR pathway plays a role in brain growth, disorders concerning brain overgrowth and growth of other tissues such as the kidney[93] [94]. Key player this pathway is mTOR, an essential kinase component of two complexes; mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). Two best known substrates of mTORC1 are ribosomal protein S6 kinase 1 (S6K1) and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1). Activation of mTORC1 leads to phosphorylation of both of these substrates. This activates S6K1 and inhibits 4E-BP1, which regulates initiation of translation and with that the synthesis of proteins. mTORC2 mainly activates mTORC1. There are many other factors known that can activate this pathway, for example, epidermal growth factor (EGF)[95], the protein sonic hedgehog (SHH)[96] and calmodulin (CaM)[97], but also IGF-1 and insulin[98]. Upon activation, AKT phosphorylates tuberous sclerosis complex 2 (TSC2). Together with TSC1 and TBC1D7, TSC2 forms the TSC complex[99]. AKT activation inhibits the TSC complex by phosphorylation of TSC2. TSC in turn inhibits Ras homolog enriched in brain (Rheb), a fundamental activator of mTORC1. Rapamycin is the main inhibitor of mTORC1 activity by binding the intercellular receptor of mTOR (FKBP12). This complex than binds the FKBP12-Rapamycin binding domain of mTOR suppressing the activation of mTOR[100].

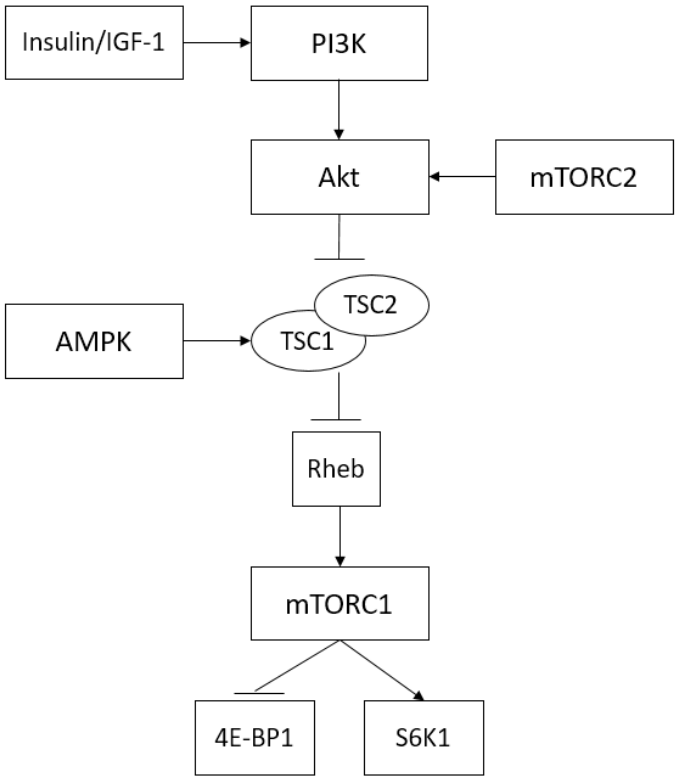

Figure 3. Schematic representation of the mTOR pathway. Stimulation of the insulin/IGF-1 signalling pathway inhibits the tuberous sclerosis complex (TSC1, TSC2), while AMP-activated protein kinase (AMPK) stimulates this complex. Rheb is an essential mTORC1 activator and is inhibited by TSC. Activation of mTOR complex (mTORC) 1 results in inhibition of Eukaryotic translation initiation factor 4E-binding protein (4E-BP) 1 and stimulation of Ribosomal protein S6 kinase (S6K) 1. mTORC2 stimulates Akt and thereby stimulates mTORC1.

The mTOR pathway during aging

As mentioned before, mTOR is involved in the regulation of longevity, making it a target to battle aging. During aging, mTORC1 signalling activity changes. However, both increased and decreased mTORC1 signalling have been found during aging. While this is also a matter of tissue and gender specificity, different studies have found opposing results in levels of phosphorylation by mTOR levels in the same tissues. Overall, mTOR does not seem to be hyperactive during aging. This indicates that even regular activity of mTOR leads to age-related diseases and pathologies, since inhibiting mTOR activity has beneficial effects on aging, health span and lifespan[101]. Another explanation could be that during aging, mTORC1 activity is de-regulated in a specific tissue which has not been identified, but which is crucial for the aging process.

The mTOR pathway and lifespan extension

Inhibition of mTOR signalling has been found to prolong lifespan. Impairing the function of mTOR leads to extended lifespan in mice. Mice with two mTOR alleles that are hypomorphic (mTOR Δ/Δ) show less activity of mTORC1 and mTOR2. As a result these mice live longer compared to mice with normal functioning mTOR alleles. mTOR hypomorphic mice are smaller than WT mice, but they do not show relative differences in food intake, glucose homeostasis or metabolic rate. In accordance with the extended lifespan, in mTOR Δ/Δ mice a reduction of biomarkers for aging tissues was found. This indicates that impairment of mTOR results in lifespan extension by affecting function of tissues and organs which normally declines during aging[102].

Other impairments of the mTOR pathway have also been found to increase lifespan. In a mouse model for nuclear lamina disorder associated with dystrophy, injection with rapamycin improved function of skeletal muscle cells, improved glucose tolerance and insulin sensitivity. Treatment with rapamycin inhibits mTORC1 and S6K1 activity, which eventually results in extended lifespan compared to mice without rapamycin treatment[103]. Moreover, S6K1 depletion can phenotypically copy the effects of rapamycin in mice . S6K1 depleted female mice have increased lifespan compared to WT mice. S6K1 depletion resulted in more resistance to age-related pathologies, such as immune dysfunction and insulin resistance in both male and female mice. Furthermore, depletion of S6K1 shows gene expression compositions comparable to CR[104]. In other words, the beneficial effects of rapamycin or CR on aging can be copied by reducing the activity of S6k1 in mice.

Besides inhibition of mTOR signalling by rapamycin, the mTOR pathway can also be regulated by the TSC complex. Through Akt-mediated activation, this complex can inhibit mTORC1 signalling[105]. In TCS1 transgenic mice mTORC1 signalling had been reduced in various tissues except in the brain. Contrastingly, mTORC2 signalling showed to be enhanced in this mouse model. This indicates that mTORC2 is stimulated by feedback mechanisms after reduced mTORC1 signalling. Moreover, TSC1 transgenic mice seemed more protected against cardiac hypertrophy, fibrosis and inflammation during aging. As with S6K1 depletion, lifespan extension could only be demonstrated in female TSC1 transgenic mice[106]. A more downstream target of the mTOR pathway, metabolic transcription factor C/EBPβ-liver-enriched Inhibitory Protein (LIP) is also involved in the regulation of lifespan. mTORC1 stimulates C/EBPβ-LIP and during normal aging an increase of LIP levels can be observed. Reduction of C/EBPβ-LIP expression, on the other hand, delays age-associated pathologies development in mice. Again only in female mice, extended lifespan is observed after genetic reduction of C/EBPβ-LIP[107]. In conclusion, most strategies of extending lifespan via mTOR signalling alterations, show promising effects on delaying the aging process. Many strategies also demonstrate lifespan extension via these alterations, however only in female mice.

Discussion

One way or another, the sirtuin, the insulin/IGF-1 and the mTOR pathway are important for the process of aging. Many alterations of either of the pathways have been demonstrated to delay the aging process or to extend health span. Alterations of the IIS pathway show that lifespan extension is strongly associated with insulin sensitivity and overall reduction of the activity of this pathway, which can also be seen under CR conditions[108], indicating reduced IIS can mimic the effects of CR. GH reduction, on the other hand, also contributes to lifespan extension. Furthermore, reducing IIS is a potential target for the treatment of cancer via Foxo1. Pharmacologically, metformin treatment results in extension of lifespan. Alterations of the sirtuin pathway sirtuins in mice have been extensively studied in relation to lifespan. Sirt6 overexpression shows a significant extension of lifespan of WT mice. However, this had only been demonstrated in male mice, which complicates the role of Sirt6 in lifespan extension. Overexpression of Sirt2 has also been proposed as a lifespan regulator. Lifespan extension through Sirt2 overexpression, however, had only been accomplished in genetically modified mice. This means this strategy cannot be applied in humans, due to ethical reasons. Pharmacological strategies are better applicable to humans for that reason. Pharmacologically, the sirtuin pathway can be activated by resveratrol and has promising effects on lifespan. However, resveratrol could only increase lifespan significantly in mice on a high fat diet and not of mice on a normal diet. This could indicate involvement of genes regulating energy metabolism in brown fat, since mice on a high fat diet generally have more brown fat than mice on a normal diet. Alterations of the mTOR pathway show that impaired activity of different players in the mTOR pathway results in extended lifespan. However, almost all these studies only showed lifespan extension in female mice, suggesting mTOR-mediated lifespan extension is extremely sex-specific and relies on either the presence of female hormones or the absence of male hormones. Also in mTOR pathway studies, genetic modification models are often used to investigate lifespan, which are not applicable in humans. Pharmacological treatment with rapamycin has beneficial effects on lifespan, however, long term treatment in humans might be difficult since rapamycin has severe side effects[109].

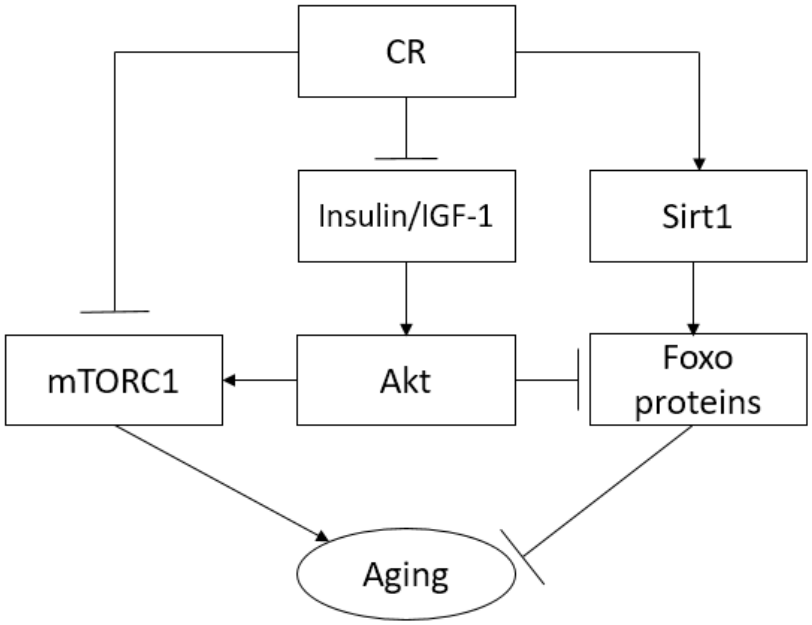

Figure 4. Schematic representation of the interactions of the different metabolic pathways and their effects on aging. Calorie restriction (CR) inhibits the aging process by inhibiting mTOR complex (mTORC) 1 and insulin/IGF-1 signalling and by stimulating Sirt1 (and Sirt 6). Sirt1 in turn stimulated several Foxo protein with aging process delaying characteristics. Inhibiting of IIS decreases the inhibiting effect of protein kinase B (Akt) on Foxo proteins and the stimulating effect of Akt on mTORC1.

Even though about two decades ago, mutations of the daf-2 gene, an orthologue of the IGF1R which can be found in C. elegans, has been shown to double lifespan of C. elegans [110]. Twenty years later, the underlying mechanisms are still not clearly understood in rodents. This is partly due to the difficulty of executing experimental setups, because of the relative long lifespan of more complex species. Furthermore, studies with C. elegans models are easier to perform, since mice have more complex interactions of each of the pathways. However, mice are more similar to humans than C. elegans, thus results from mouse model studies are more representable for humans. The pathways discussed in this assay have many overlapping targets and stimulating one, almost inevitably also effects the other pathways. When targeting the IIS pathway, for example, the mTOR pathway is effected as well. This is mostly mediated by Akt, since Akt can also activate mTORC1. Furthermore, Akt also inhibits several Foxo proteins, linking IIS to sirtuin signalling. Although much is known about the pathways and how they work in theory, when looking at the pathways in vivo, different outcomes than expected are often found. Because of many other interacting factors that function as feedback loops. The same goes for the interpretation of mouse model results to human. The fact that a strategy proved to be effective in mice, could lead to a different effect in humans. For example, due to IGF-1 levels decreasing in humans during aging, but stay rather constant in mice, which could give different results when targeting the IGF-1 signalling pathway in humans. Nonetheless, mouse model experiments are extremely useful for studying the mechanisms behind different metabolic pathways and the interaction of different organs and tissues, which cannot be investigated using cell lines. By working out these mechanisms, targets more downstream of these pathways could be found providing more specific effects and with that less adverse effects. Figuring these mechanisms out in more detail could also provide targets for delaying the aging process and the development of age-associated diseases, instead of treating each disease separately. Investigating the role different organs and tissues in these pathways, could help explain why sometimes alterations of these pathways show contradicting results.

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