



university of
 groningen

University of Groningen
Faculty of Science and Engineering

Bachelor Thesis in Biomedical Sciences

Aging and Dieting

An overview of dietary interventions and their role in lifespan and health span

Author:

Renzo Boersma
s2611236

Supervisor:

dr. C. Müller

19 July 2017

Academic Year 2016/2017

Contents

Introduction	p. 2
Pathways involved in aging	p. 3
• Sirtuins	p. 3
• Insulin/insulin-like growth factor signaling pathway	p. 5
• Mechanistic target of rapamycin	p. 6
Dietary interventions	p. 8
• Calorie restriction	p. 8
• Low-protein – high-carbohydrate diet	p. 10
• Amino acid restriction	p. 12
• Circadian rhythms and time-restricted feeding	p. 13
Dietary restriction mimics	p. 15
Modulation of the microbiome	p. 17
Conclusion and future perspectives	p. 19
Acknowledgements	p. 20
References	p. 20

Introduction

Everybody wants to get old and stay healthy but with aging, age associated diseases become more frequent. Age associated diseases are a growing problem, due to increased food intake and less physical activity. The incidence of obesity and metabolic disorders is a worldwide problem, especially in Western society with abundant food availability. It was shown by Sturm & Hattori (2013) that the morbid obesity rates continue to rise rapidly in the United States, leading to elevated rates of diabetes, hypertension, dyslipidemia, and other cardiovascular disease factors. Not only the quality of life is less for these individuals, the economic impact is just as relevant (Apovian, 2013).

Over the past decades, research has given us a better insight in the importance of maintaining a healthy and balanced diet. But what exactly does a healthy and balanced diet mean, and what are the consequences of deviating to this balanced diet? And what are the effects of nutrient intake on aging? As of now, these are questions without a clear answer, but are necessary to devise effective public health policy in the battle against obesity and other metabolic disorders (Simpson & Reubenheimer, 2012).

The relationship between food intake and aging is a hot topic. Cells age, ultimately leading to aging of tissues and organs, and is detrimental in the end. To see whether aging is influenced by nutritional intake; aging research has provided us with better understanding of genetic control of aging. This can help to look for possibilities to extend health span and lifespan using dietary interventions. Particular nutrient sensing pathways are involved in lifespan control and delaying or completely eliminating the change of developing age-related diseases is heavily investigated (Piper & Bartke, 2008).

The present thesis aims to investigate whether dietary interventions have beneficial effects on extending health span and lifespan, and which molecular mechanisms are involved and whether they are applicable for humans. The first chapter discusses three important pathways involved in the process of aging; the sirtuin family of NAD⁺-dependent enzymes, the insulin/insulin-like growth factor signaling pathway, and the mechanistic target of rapamycin. The second chapter will discuss dietary interventions and their effects on aging. Dietary interventions that will be discussed are calorie restriction, low-protein high-carbohydrate diets, amino acid restriction with extra attention for methionine restriction, and time-restricted feeding. The third chapter points out whether medicines can be used to mimic the results of dietary interventions, without having to comply with the diet itself. The drugs described in this thesis are Metformin, Rapamycin and Resveratrol. The fourth and last chapter elucidates the role of the intestinal microbiome in relation to both the process of aging as well as dietary interventions.

Pathways involved in aging

Sirtuins

Silent information regulators (sirtuins) are a family of NAD⁺-dependent enzymes and are involved in nutrient sensing. (Hall et al, 2013). Sirtuins act as metabolic sensors by detecting fluctuations in the NAD⁺/NADH ratio. They are activated when nutrients such as glucose decrease, while NAD⁺ accumulates. In mammals, 7 different sirtuins are known (Frye, 2000). The evolutionarily conserved sirtuins show many similarities, but also significant differences. Differences are found in the distinct expression patterns, catalytic activities and biological functions, as well as in the localization in the cell (Houtkoper et al, 2012). Among the mammalian sirtuins, the most is known about SIRT1 in regard to aging. Only SIRT1 will therefore be discussed.

Increased expression of SIRT1 is known to be able to extend lifespan, delay aging and prevent aging-associated diseases. The main function is catalyzing the deacetylation of histones and some non-histone proteins (Ramis et al, 2015). The enzyme is involved in many processes. In vitro experiments have shown that up regulation of SIRT1 attenuates insulin-like growth factor-1 (IGF-1)-induced primary fibroblasts senescence via p53 acetylation (Tran et al, 2014). Senescence is one of the hallmarks of aging. In vivo experiments in mice have shown that SIRT1 activators such as SRT1720 can augment activity, leading to attenuation of stress-induced premature cellular senescence (Yao et al, 2012). Over expression of SIRT1 in the liver increases expression of PPAR α and activates PGC-1 α , resulting in improvements in oxidative metabolism and regulations of lipid metabolism in response to nutrients (Purushotham et al, 2009). Enhancement of SIRT1 expression was shown to be beneficial against onset of Alzheimer's disease as well in mice (Sweeney & Song, 2016).

With aging, levels of SIRT1 in the body decline, due to impairment at both transcriptional as well as translational level. This is seen in the brain, liver, skeletal muscle and white adipose tissue. The reduced endogenous level of SIRT1 upon aging is probably related to the development of age-related diseases; since whole body over expression of SIRT1 improves several metabolic parameters and delays the onset of metabolic diseases. Repressed expression of SIRT1 in the brain is linked to a causative role in cognitive decline and neurodegeneration in aging mice (Cho et al, 2015)

A role for improving life span and aging-related diseases through SIRT1 can be found in increased mitochondrial biogenesis mediated by SIRT1. This is important because mitochondrial dysfunction increases upon aging. Both PGC-1 α -dependent and PGC-1 α independent pathways seem to play prominent roles. I will discuss the PGC-1 α -dependent pathway shortly, shown in figure 1. Mitochondrial biogenesis is promoted by SIRT1 via PGC-1 α and occurs through the induction of mitochondrial genes (both nuclear and mitochondrial encoded). SIRT1 is able to activate PGC-1 α by deacetylation,

and PGC-1 α in turn induces nuclear encoded mitochondrial genes. Deacetylated PGC-1 α can also interact and collaborate with TFAM which is an important regulator of mitochondrial gene expression of mitochondrially encoded genes and of mitochondrial DNA replication. Consequently, SIRT1 mediated deacetylation of PGC-1 α transcription and replication of mtDNA, which ultimately increases mitochondrial biogenesis (Aquilano et al, 2013).

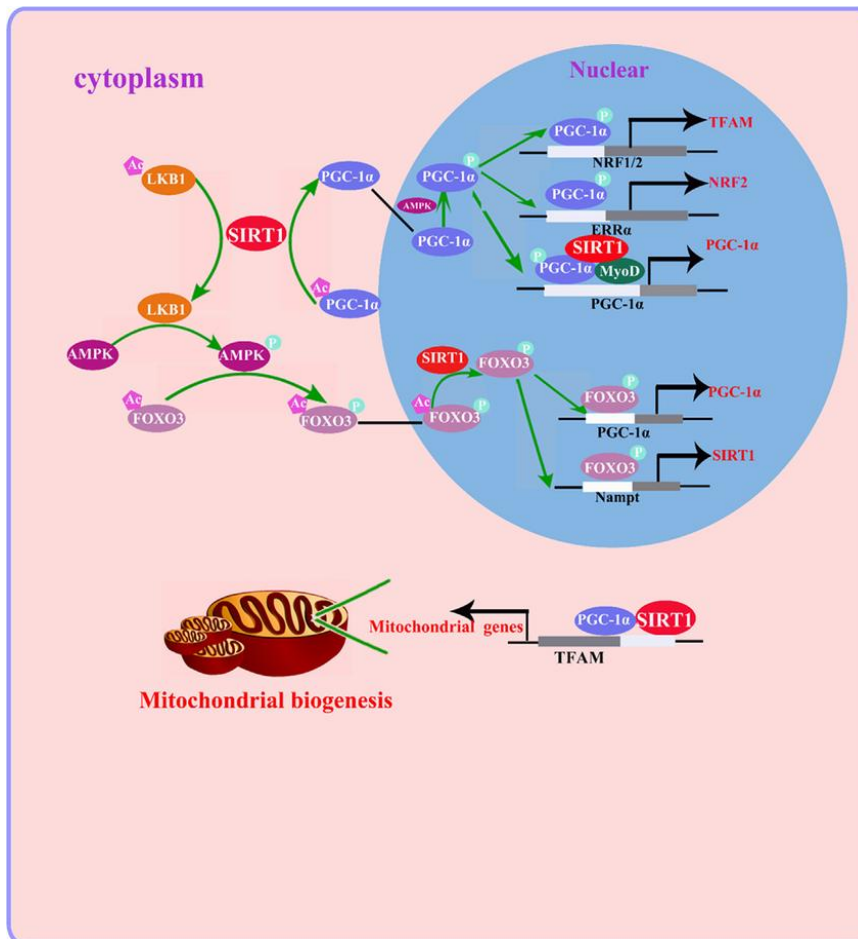


Figure 1. Control of mitochondrial biogenesis by PGC-1 α (Yuan et al, 2016).

In figure 1, more pathways are shown. SIRT1 is able to modulate the nutrient sensor kinase AMPK as well. Reduced AMPK activity is involved in several aging-associated processes, such as insulin resistance, obesity, and deterioration of mitochondrial biogenesis (Okazaki et al, 2015) LKB1, an AMPK-activating protein kinase, is activated by SIRT1, and promotes phosphorylation and activation of the catalytic α -subunit of AMPK.

Another essential role in the SIRT1-PCG-1 α pathway during the regulation of mitochondrial biogenesis is fulfilled by FOXO3, as seen in figure 1. During accelerated aging in cardiac microvascular endothelial cells and in the heart of ischemic reperfusion rats, which have elevated ROS production and disorder of mitochondrial homeostasis, down regulation of the expression of SIRT1 and FOXO3 is observed (Lin et al, 2014). SIRT1 is able to deacetylate and activate FOXO3, which in turn is found to

induce transcription of PGC-1 α which thereby further contributes to the activation of mitochondrial genes, and the enzyme Nampt. Enhancement of NAD⁺/NADH ratio mediated by Nampt, is required for SIRT1 dependent deacetylation and causes an activation of SIRT1 inside the cell. There is positive feedback SIRT1-FoxO3-Nampt-SIRT1 activity.

Insulin/insulin-like growth factor signaling pathway

The insulin/insulin-like growth factor signaling pathway is another essential nutrient sensing pathway, which is activated by insulin and other growth factors. Many similarities are seen between invertebrates and mammals in this pathway; however one insulin-IGF-1 receptor is present in invertebrates, where mammals have 3. Mammals also have 3 different insulin/IGF tyrosine kinase receptors. After the ligand binds to the receptor, the activated receptor phosphorylates several intracellular substrates, such as IR substrates and Shc. These phosphorylated substrates have specific docking sites for intracellular effectors, leading to the activation of the two signaling pathways; the PI3K-PKB/ATK pathway and the Ras-MAPK pathway, as seen in figure 2. The main difference is that the PI3K-PKB/AKT pathway regulates most of the metabolic effects of insulin/IGF-1 signaling, whereas the Ras-MAPK pathway regulates most of the mitogenic effects of insulin/IGF-1 signaling (Taniguchi et al, 2006).

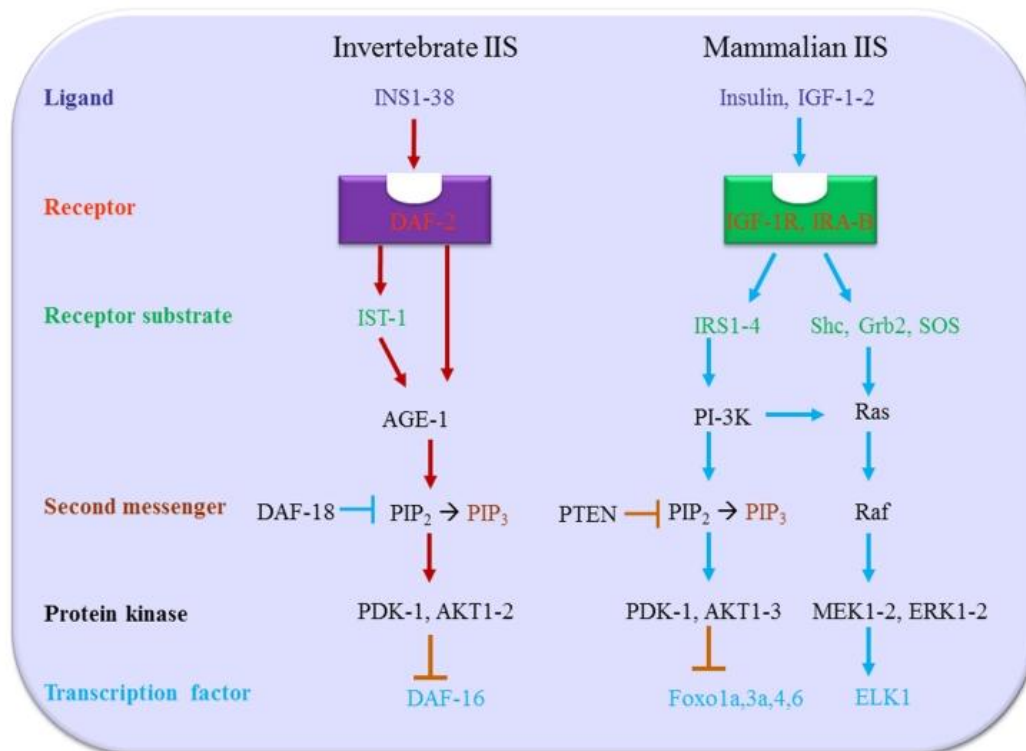


Figure 2. Simplified description of the insulin/IGF-1 signal transduction (IIS) pathway in invertebrates and mammals (van Heemst, 2010).

FoxO transcription factors are important downstream mediators of this pathway. AKT phosphorylates FoxO proteins and thereby keeps them inactive in the cytoplasm. When the pathway is inactive (low carbohydrates/ low insulin) the phosphorylation of FoxO is reduced. Unphosphorylated FoxO can move to the nucleus and transactivate target genes (metabolic and stress response genes). Of FoxO, four different members have been identified, and six distinct IRS proteins (Cai et al,2003; Horst van der & Burgering, 2007). Now that it is known that different forms and isoforms exist, it has increased the possibilities for tissue specificity and fine-tuning of IIS transduction under various physiological conditions.

With aging, insulin sensitivity declines. It has been shown in worms, flies, and mice, that there are associations between reduced insulin/IGF-1 signaling and longevity. Speculations are that this applies for humans as well. However many studies show conflicting and controversial results. So is shown by Taniguchi (2006) that defects in insulin signaling have been associated with insulin resistance and diabetes. Defects in GH/IGF-1 signaling have been associated with defects in growth and an increased risk of cardiovascular diseases (Besson et al, 2003). However, Italian centenarian were shown to have lower IGF-1 plasma levels, because of a genotype recombination at IGF-IR and PI3KCB genes (Bonafé et al, 2003). Variants in FOXO3A showed longevity in different populations, including an ethnic Japanese population in Hawaii (Willcox et al, 2008; Anselmi et al, 2009; Flaschbart et al, 2009). It seems that variation in the latter mentioned FOXO3A is most consistently associated with human longevity. The role of IGF-1 signaling in human aging is not clarified; maybe a reduced IGF-1 signaling at a younger age helps to prevent the occurrence of age associated diseases later in life.

Mechanistic target of rapamycin

The mechanistic target of rapamycin (mTOR) is a serine/threonine protein kinase, activated by nutrients (particularly amino acids). mTOR is inhibited by rapamycin, which is a compound produced by bacteria, that inhibits proliferation of eukaryotic cells (Vézina et al, 1975) mTOR is found in two distinct complexes, each complex contains distinct protein components and phosphorylates different substrates. Most is known about the mTORC1 complex, which is acutely inhibited by rapamycin. Both complexes regulate processes required for cell growth and metabolism. mTORC1 controls ribosomal biogenesis, protein translation, and autophagy while the mTORC2 complex has been primarily characterized as a downstream effector of the insulin/IGF-signaling pathway (Kennedy & Lamming, 2016).

mTORC1 activity is linked to the IIS pathway through multiple connections, seen in figure 3. mTORC1 is activated by IIS through AKT, and can negatively affect IIS through S6Kinase in a feedback mechanism, which inhibits insulin receptor substrate 1 (IRS-1). This feedback mechanism is believed to contribute to insulin resistance upon hyper

activation of mTORC1 (Takano et al, 2001). Interaction via the two pathways also happens via mTORC2, which activates AKT to repress FOXO1 and FOXO3 in mammalian cells (Guertin et al, 2006). Experiments have shown that mTORC1 modulates aging by mechanisms that overlap but are distinct from IIS. mTORC1 seems to mainly act downstream in the IIS pathway (Hansen et al, 2007; Pan et al, 2007).

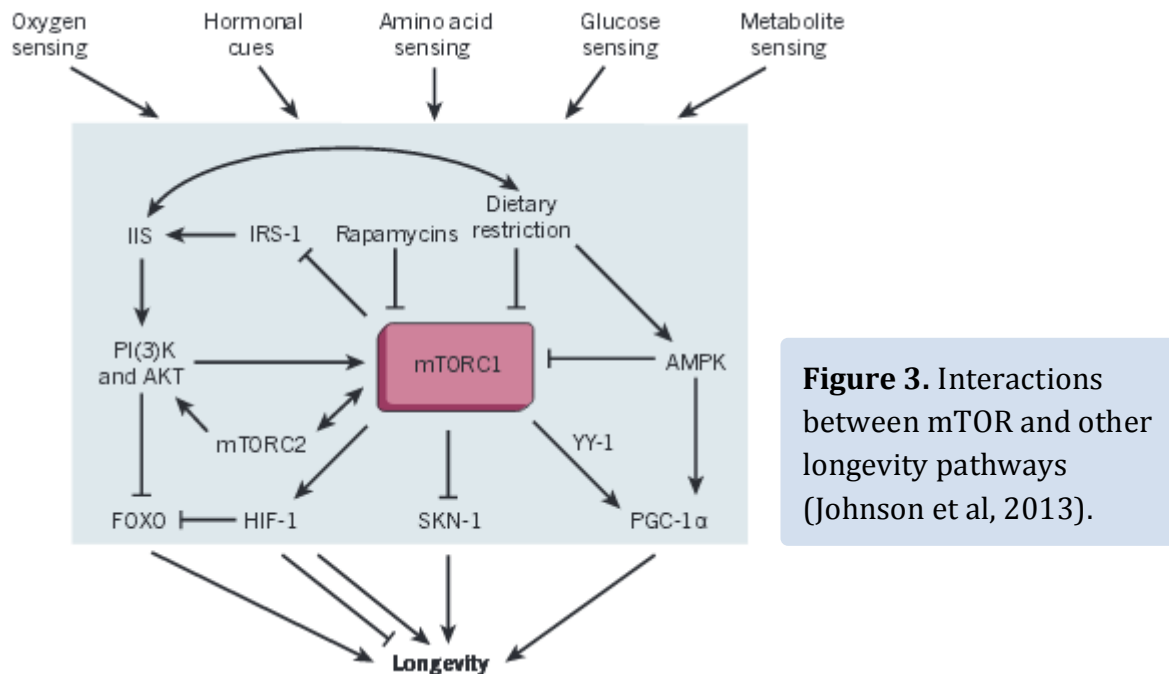


Figure 3. Interactions between mTOR and other longevity pathways (Johnson et al, 2013).

Another important molecule in mTOR signaling is AMPK, a conserved sensor of energy status that is activated in response to low ATP levels, resulting in negative regulation of mTORC1 (figure 3.). In both *C. elegans* and short-lived, cancer-prone strains of mice, over expression of AMPK can extend lifespan (Apfeld et al, 2004; Onken & Driscoll, 2010; Anisimov et al, 2010). AMPK inhibits mTORC1 through at least two distinct mechanisms; AMPK phosphorylates TSC2 on conserved serine sites, resulting in activation of TSC2 and down regulation of mTORC1 activity, and AMPK directly phosphorylates The mTORC1 subunit raptor to impair mTORC1 signaling (Inoki et al, 2003; Gwinn et al, 2008). The actual situation however is more complex because both AMPK and mTOR interact with multiple additional factors (Mair et al, 2011).

The relationship between mTOR signaling and aging was first demonstrated in worms and flies, where genetic inhibition of mTOR signaling could extend lifespan (Kapahi et al, 2004; Vellai et al, 2003) Rapamycin treatment extends lifespan in yeast, worms, flies and even in mice (Bjedov et al, 2010; Harrison et al, 2009; Robida-Stubbs et al, 2012). Mouse models with decreased mTOR signaling, which has deletion of S6K1, expressing a hypomorphic allele of MTOR, or mice heterozygous for both mTOR and mLST8, possessed extended longevity (Lamming et al, 2012; Selman et al, 2009) Conversely, signaling of mTORC1 is increased in many age-related diseases and pathologies, including cancer (Bar-Peled et al, 2013; Grabiner et al, 2014).

Whether mTORC1 signaling increases during healthy aging is debatable. Some studies suggest that mTORC1 substrate phosphorylation increases with age in individual rodent tissues (Chen et al, 2009; Sengupta et al, 2010). Other studies show that with aging mTORC1 signaling decreases or increases depending on the tissue (Baar et al, 2016). It should be noted that although there is an increase in some tissues during aging, it never becomes hyperactive. The effect of rapamycin on lifespan suggests that even normal levels of mTORC1 signaling may be inappropriately high for the maintenance of health in aging cells and tissues (Blagosklonny, 2009). Not only does rapamycin extend lifespan, it also prevents or delays the onset of aging-related diseases such as cancer and Alzheimer's disease, rejuvenates the aging mouse heart, and ameliorates age-related cognitive decline (Dai et al, 2014; Spilman et al, 2010; Wilkinson et al, 2012). Besides the promising positive effects of rapamycin, negative effects such as immunosuppression, and metabolic effects including hyperlipidemia and decreased insulin sensitivity in humans are seen (Lamming et al, 2013; Arriola Apelo et al, 2016).

Dietary interventions

Calorie restriction

Calorie restriction is the reduction of energy intake without malnutrition, and is to date the most successful intervention in the aging process. McCay showed in 1935 that calorie restriction retards aging and extends median and maximal lifespan. Similar findings have been shown in a variety of species including yeast, worms, flies, fish, mice and rats (López-Lluch & Navas, 2016). I will discuss the most important mechanisms involved in this dietary intervention.

Calorie restriction is basically a decrease in energy intake without malnutrition, producing an imbalance in the metabolic system. Several regulatory pathways interact to make sure that a new stable equilibrium is formed. This new equilibrium responds to the relationship between growth-associated pathways, such as the insulin-insulin/IGF-1 receptor system, sirtuins and AMPK, which can be seen in figure 4. From evolutionarily view, the mechanisms and their connections are heavily conserved between many species. When calories in the diet decrease, it activates systems involved in a more efficient metabolism, a higher protection against cellular damage and the activation of remodeling mechanisms, whereas less efficient metabolism and synthetic pathways are blocked (Testa et al, 2014; Michan, 2014).

Plasma levels of IGF-1, insulin and glucose are reduced with calorie restriction in both rodents and humans (Argentino et al, 2005; Weiss et al, 2006). CR inhibits IGF1 signaling and mTORC1 signaling and thereby inhibits protein synthesis and other anabolic pathways. FoxO proteins get activated upon reduced IGF1 signaling in response to CR. Activation of FoxO proteins is important in the effect of CR on lifespan since the

inhibition of the FoxO orthologue daf-16 in *C. elegans*, by IGF-1-dependent signaling abrogates the increase of lifespan in response to CR (Kenyon, 2010).

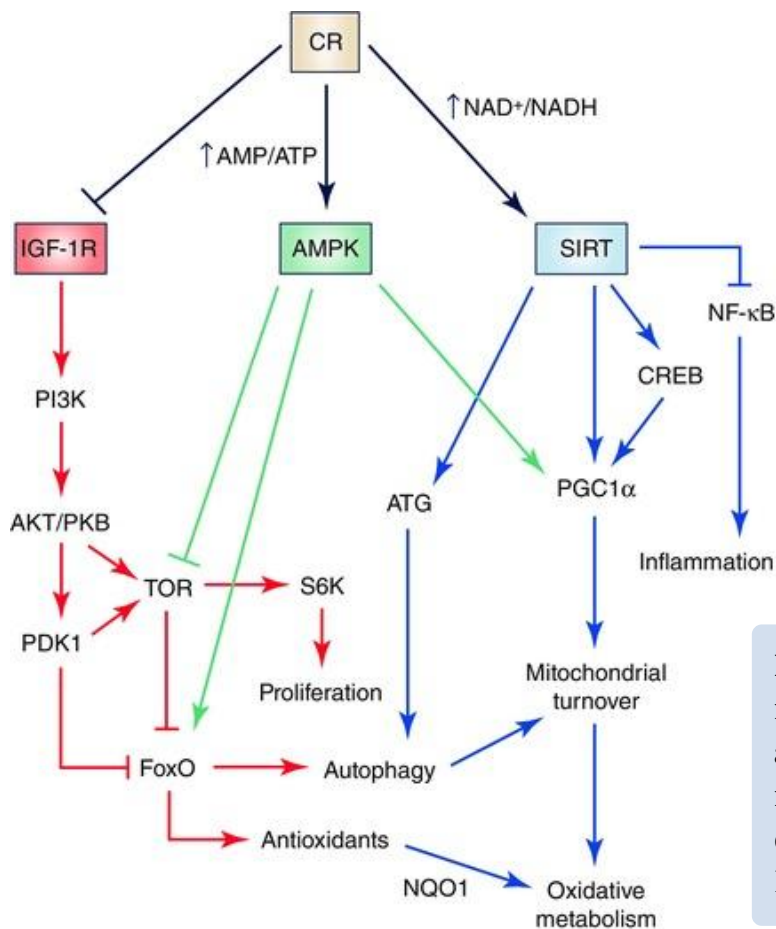


Figure 4. Complex interaction of proliferative and protective mechanisms in the CR prolongevity effect in organisms (López-Lluch & Navas, 2016).

TOR is inhibited by calorie restriction as it was demonstrated in invertebrates such as *C. elegans* and *D. melanogaster*. Down-regulation of TOR resulted in an increased lifespan in these organisms (Sharp, 2011). Studies in mice showed similar effects (Harrison et al, 2009) S6K is a protein kinase involved in protein synthesis and is activated by TOR in a wide variety of model organisms. When S6K is decreased, it extends lifespan and delays the progression of age-related diseases (Selman et al, 2009).

AMP-dependent protein kinase (AMPK) is a sensitive energy sensor in cells and is an inhibitor of TOR. When the AMP/ATP ratio increases, AMPK is activated. This event can occur when cells are deprived of glucose (Hardie, 2011). It has been demonstrated that an increase in AMPK activity is associated with a longer lifespan while inhibition shortens it (Apfeld et al, 2004; Harkness et al, 2004; Tohyama & Yamaguchi, 2010). However in response to CR, some studies showed that in mammals this kinase is not affected or reduced (Gonzalez et al, 2004; To et al, 2007). Other studies actually did describe increased AMPK activity in heart and skeletal muscle (Jager et al, 2007; Miller et al, 2012).

Sirtuins are one of the best known effectors of calorie restriction. Calorie restriction induces the expression and activity of sirtuins in many organs and their activity is associated with metabolic effects found in these organisms after the intervention (Imai & Guarente, 2010). Sirtuins play a central role in response to calorie restriction, they act as nutrient and metabolic sensors by detecting fluctuations in the NAD⁺/NADH ratio. Sirtuins are activated when nutrients such as glucose decrease, while NAD⁺ accumulates. The complexity of sirtuins in mammals, promote the idea that they can have both pro- and anti-aging capacities, depending on the cell type or conditions (Kaeberlein, 2008; Li et al, 2008).

Low-protein – high-carbohydrate diet

The previous chapter described calorie restriction; it remains debatable whether the benefits are due to the reduction of total calorie intake itself or because of a reduction in one of the macronutrients (Simpson et al, 2015; Minor et al, 2010). To better understand and resolve these issues, on dietary intervention is to reduce the amounts of each macronutrient. The biggest problem often seen with these kinds of interventions is compensatory feeding. Protein seems to have the strongest impact on food intake, such that low-protein diets will lead to an increase in food intake and vice versa, also known as protein leverages. Therefore mice that get a low protein diet will eat more food per day (Simpson & Raubenheimer, 2005).

A widely used method to try to disentangle the effects of calories and macronutrients on health and aging is the Geometric Framework (Le Couteur et al, 2016). Animals are ad-libitum fed on one of many diets, varying in macronutrients and total energy content. There is no caloric restriction and animals can do compensatory feeding via increasing or decreasing intake of that particular diet. The Geometric Framework can now be used to give insight in outcomes such as lifespan across a dietary landscape or different macronutrient concentrations, macronutrient ratios and calorie content. Figure 5 clearly shows that ad-libitum-fed diets lower in protein and higher in carbohydrate are associated with longer lifespan. Interestingly, diets with higher protein and lower carbohydrate are associated with improved reproductive outcomes (Holliday, 2006).

Little is known about the mechanisms linking LPHC diets and its role in aging. It was seen in mice on low-protein high-carbohydrate diets that they showed a small reduction in phosphorylation of hepatic mTOR which was correlated with lower circulating branched chain amino acids and higher glucose levels (Solon-Biet et al, 2014). Mice on this dietary intervention also had the lowest insulin levels in the blood stream in spite of the higher carbohydrate intake. Effects of low-protein high-carbohydrate diets on AMPK and/or sirtuins have not yet been reported. Another mechanism, involving the hormone FGF21 might be involved in the beneficial effects of the low-protein high-carbohydrate diets. FGF21 has been found to be influenced by dietary protein and has many

downstream effects on metabolism and mitochondrial function that would be expected to influence aging (Solon-Biet et al, 2015).

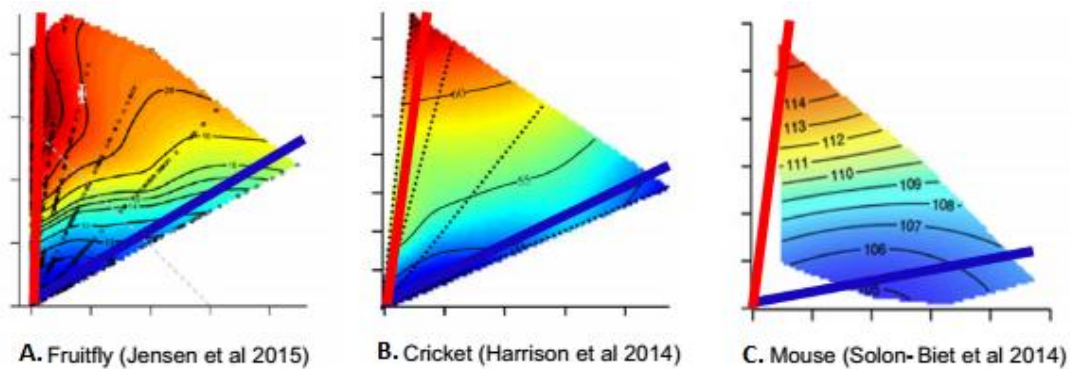


Figure 5. Response surfaces for lifespan versus dietary macronutrients. The x axis represents a measure of protein and the y axis represents a measure of carbohydrates. The red line represents the nutritional rail or PC ratio associated with the longest lifespan while the blue line represents that with the shortest lifespan.

Different studies have compared the calorie restriction diet to the low-protein high-carbohydrate diet, to see if there are similar metabolic outcomes. In an eight week study on mice, LPHC fed mice had similar metabolic improvements as seen under CR, without development of increased body adiposity, which is often observed in chronic long term LPHC feeding (figure 6). Manipulating P:C ratios under CR conditions did not create any additional benefits, nor were there detrimental effects to the mice (Solon-Biet et al, 2015). LPHC mice did show increased energy expenditure, consistent with increased diet-induced thermogenesis serving to dissipate excess ingested energy and slow development of adiposity (Huang et al, 2013; Stock, 1999). When subjects are exposed

to LPHC for a longer time, there is an association with increased body weight, adiposity and fatty liver, indicating that mechanisms become less effective over time (Huang et al 2013; Solon-Biet et al, 2014; Sorensen et al, 2008).

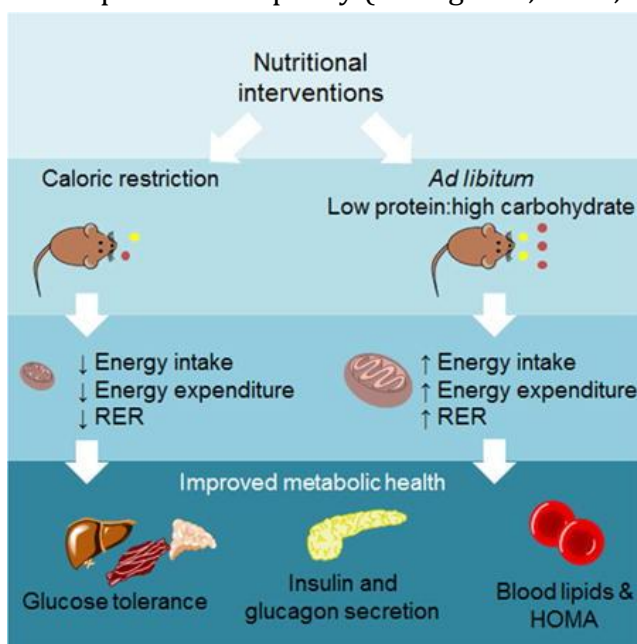


Figure 6. Comparison between two dietary interventions; caloric restriction vs. ad libitum low-protein high-carbohydrate (Solon-Biet et al, 2015).

Amino acid restriction

Amino acids are the building blocks for protein synthesis, but play roles in other essential metabolic processes as well. To give some examples; methionine is required for one-carbon transfer reactions, tryptophan is a precursor for NAD and serotonin biosynthesis, glutamate acts as a neurotransmitter, and other amino acids can serve as intermediate metabolites in a variety of processes ranging from gluconeogenesis to anaplerosis in the citric acid cycle. Mammals are unable to synthesize all amino acids themselves, so that they must acquire the essential amino acids through dietary means. Amino acid restriction is a dietary intervention, which seems to be able to extend longevity (Gallinetti et al, 2013).

Two general amino acid control pathways are known, shown in figure 7. They activate GCN2 kinase and the target of rapamycin (TOR) kinase pathway. GCN2 is activated in response to amino acid deprivation, upon binding of uncharged tRNAs. GCN2 phosphorylates the eukaryotic initiation factor 2a (eIF2 α) and thereby inhibits general protein biosynthesis. The TOR pathway senses the presence of particular amino acids, which activates a Rag heterodimer at the lysosomal surface, which in turn recruits and activates mTORC1. Thus amino acid deprivation inhibits mTORC1 through Rag inhibition. Inhibition of mTORC1 happens through GCN2 activation as well. Inactivation of mTORC1 results in dephosphorylation of the ribosomal protein S6 kinase (S6K) and the eukaryotic translation initiation factor 4-(eIF4)-binding protein (4EBP). Consequently, both the GCN2- and TOR-dependent pathways lead to reduction of global protein synthesis (Gallinetti et al, 2013).

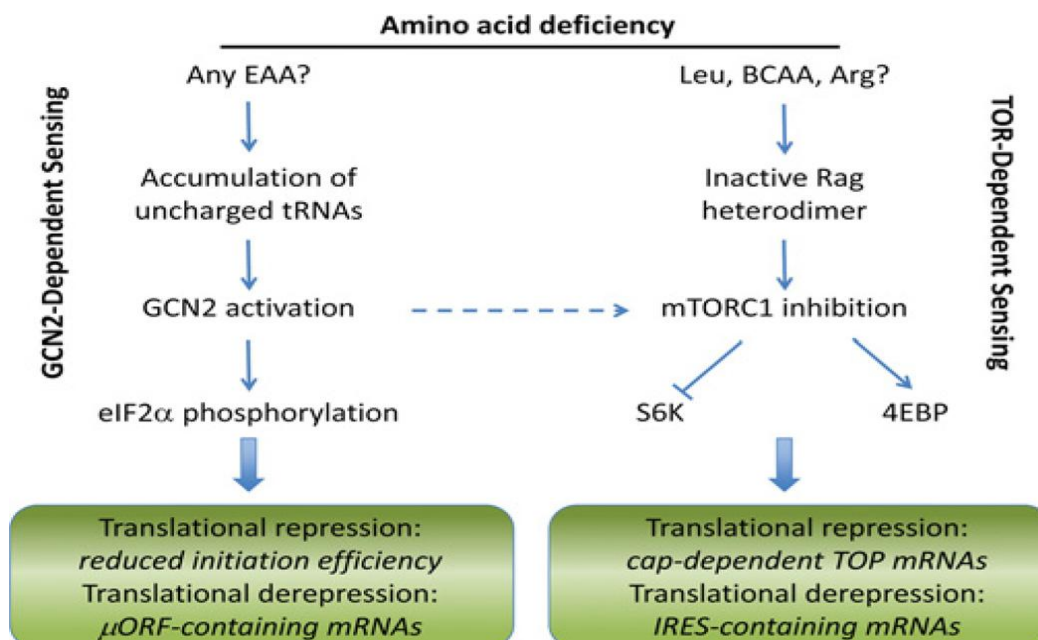


Figure 7. Integration of amino acid sensing with translational control (Gallinetti et al, 2013).

Model organisms have shown that deficiency of downstream targets of TOR, such as S6K or translation factors/regulators extend lifespan (Kaeberlein & Kennedy, 2007). Under conditions of impaired TOR, there is improved activation of autophagy, a catabolic process that enhances degradation and recycling of damaged cellular components during aging, contributing to longevity. Regulation of autophagy in response to nutrient starvation, involves not only TOR but GCN2 signaling as well (Tallóczy et al, 2002). Based on these findings, amino acid restriction can extend lifespan.

So far, we've only discussed the effects of total amino acid reduction, but it is also possible to look at reduction of certain amino acids. Dissecting the roles of dietary amino acids could possibly show which ones are the most important in extending lifespan. So far, the most successful intervention has been methionine restriction. Positive effects have been seen in different model organisms, such as fruit flies, mice, and rats, but its beneficial effect in humans is not known. Research on mice with low methionine consumption led to extended lifespan compared to those with increased dietary intake (Orentreich et al, 1993). Another study showed that when 12 month old mice were put on a low methionine diet, they showed both maximum and median lifespan extension (Sun et al, 2009). Besides lifespan extension, other benefits were seen. Obese mice on a low methionine diet were rescued from severe steatosis and significantly reduced triglyceride, serum alanine aminotransferase and aspartate aminotransferase, and plasma insulin levels (Malloy et al, 2013). In Wistar rats, ROS levels decreased in multiple tissues and organs when on a methionine restricted diet (Sanchez-Roman et al, 2011; Sanz et al, 2006; Caro et al, 2008; Caro et al, 2009). It is commonly accepted that low ROS levels contribute to maintaining a healthy lifespan. *Drosophila* did not show any benefits with methionine restriction (Troen et al, 2007; Lee et al, 2014).

Circadian rhythms and time-restricted feeding

An important aspect of not only the body, but also individual cells, is the circadian clock. The clock is roughly 24 hours, and is regulated by the suprachiasmatic nucleus in the hypothalamus, which is composed of a network of neurons which intricate intercellular communication to produce outputs through both neural and humeral cues (Welsh et al, 2010; LeSauter & Silver, 1998). Not only does the SCN receive internal information, external factors such as light can help the organism to coordinate with their environment; a day-night rhythm. Interesting for this thesis, is that nutrients and the circadian clock are in a close relationship.

Insulin and glucagon are regulated by the internal clock. Insulin is produced by β -islets in the pancreas. Production of insulin is normally at its lowest around 17.00h and peaks around 04.00h in humans (Goel et al, 2009). Both feeding-fasting patterns and circadian rhythms control hormonal release of insulin. Nutrient levels in the blood are greatly

influenced by eating patterns and can therefore act as an acute overriding signal. Insulin and glucagon are modulated by the circadian rhythm, controlling production and secretion at the cellular level (Kalsbeek et al, 2008; Sadacca et al, 2011). Sadecca (2011) showed in vitro, that β -islet cells exhibit robust rhythms of both Bmal1 and Per1. Bmal1 knockout in the pancreases resulted in disrupted glucose homeostasis and insulin release despite displaying normal activity and feeding-fasting rhythms. This illustrates the significance of the molecular clock at cellular and tissue level on physiology.

Cortisol is a steroid hormone in the glucocorticoid family that is involved in metabolism and stress response. The hormone is produced and secreted rhythmically and regulated by the hypothalamus pituitary axis and the autonomic nervous system. Not only are glucocorticoids release every 24h with the circadian rhythm, they have 1-2h ultradian rhythms as well. Peak levels synchronize with the beginning of the active phase to aid in arousal; at early morning in diurnal animals and at early night in nocturnal animals. Glucocorticoids influence circadian rhythms by feeding back on the clock; they bind to glucocorticoid response elements (GRE) in the Per1/2 and Rev-Erb α/β promoter, to activate and suppress transcription respectively (So et al, 2009; Torra et al, 2000; Yamamoto et al, 2005).

Circadian rhythms dampen with age. One of the most obvious consequences of disruption in humans can be observed in activity-rest cycles (Dijk et al, 2001). Circadian disruption does further increase the risk of developing cancer, cardiovascular diseases, obesity, immune disorders, infertility, and affective disorders (Hastings et al, 2003). Mice deficient in BMAL1 (the core component of the circadian clock), demonstrated early onset of aging, including a decrease in muscle and subcutaneous fat, cataracts, and organ shrinkage. (Kondratov et al, 2006). Shift workers show similar abnormalities, their circadian disruption is associated with cardiovascular disease, metabolic disorders, and cancer (Kamdar et al, 2013; Proper et al, 2016; Vyas et al, 2012).

It is known that daily eating patterns can affect the amplitude and phase of circadian rhythms (Asher & Sassone-Corsi, 2015). So far, this thesis only discussed dietary interventions based on reductions. Time restricted feeding is another dietary intervention that does not require reduction of calories or any nutrient. TRF is based on circadian biology to allow the body a daily fasting period, in which only water is allowed. TRF in combination with high-fat diet has been powerful in elucidating the effect of eating pattern on the prevention and treatment of metabolic disease. It is possible to induce obesity in model organisms on a high-fat diet. These so called diet induced obesity (DIO) animals are a simple model of obesity without genetic disruption. The diet changes eating pattern in mice, so that they continuously snack on the high-fat diet throughout day and night. Consequence of random eating patterns is disruption of the circadian oscillator in metabolic organs including the liver, resulting in disease. When mice on high-fat diet are granted access to food for 8, 9, 12, or 15 hours during the nighttime, they consume the same amount of total daily calories as the ad libitum fed counterparts. The difference is that these TRF mice are largely protected from metabolic

diseases. Even mice with pre-existing obesity due to being ad libitum fed on a high-fat diet, benefit from the therapeutically effect of TRF (Chaix et al, 2014; Chaix & Zarrinpar, 2015; Sherman et al, 2012).

Positive effects of TRF have been shown in more studies. Elevated levels of the transcription factor PPAR γ , which are known to promote fatty acid synthesis, elongation, and desaturation are observed in DIO livers. TRF reduces PPAR γ expression, paralleling a reduction in long chain fatty acid and unsaturated fatty acid. The actions of increased fatty acid oxidation and reduced fatty acid synthesis cause a >50% reduction in liver fatty acids and nearly complete absence of fat droplets in hepatocytes. This explains the reduction in fatty liver disease and fibroses in TRF mice. The mice show mildly elevated levels of ketone bodies as well, which is linked to several benefits in metabolism and central nervous system function (Akram, 2013; D'Agostino et al, 2013). Another beneficial effect of TRF is alteration of white adiposity tissue function and inflammation. The size of adipocytes, macrophage infiltration of white adiposity tissue, and inflammatory cytokine production are reduced and mitochondria content is increased in these tissues (Hatori et al, 2012). Benefits are not only seen in high fat diets but also on high fructose and standard diets (Chaix et al, 2014). Probably the most interesting finding was the effect of TRF timing in relation to day or night and its effect on metabolism. Although TRF mice are always leaner than their ad-libitum fed DIO counterparts, mice with daytime access to high fat diet are worse than the night TRF. The underlying mechanisms remain unknown, and it should be highlighted that no significant differences were observed when TRF was compared to a standard diet (Manoogian & Panda, 2016).

Dietary restriction mimetics

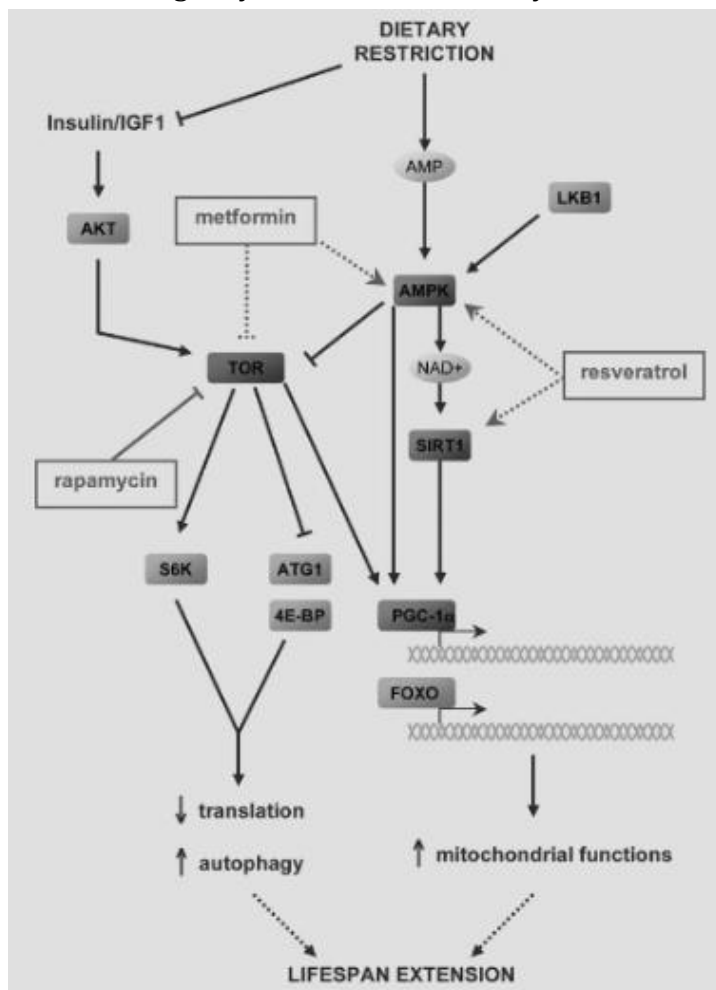
So far, this thesis has addressed the beneficial effects of caloric restriction and other dietary interventions on lifespan and health. The main problem is, to implement such restriction in our lifestyle, especially in first world countries where food is abundant. Gerontologists and biologists attempt to develop drugs to mimic the beneficial effects of dietary restriction, without the need for limitations on the diet itself. Dietary restriction mimetics are one of the possibilities. Resveratrol, rapamycin and metformin are under investigation to be used as mimetics (Lee & Min, 2013).

Resveratrol is the most studied dietary restriction mimetic and is a polyphenol compound isolated from the skin of red grapes. The compound was identified and found through screening of small molecular libraries for compounds that activate sirtuin and extend lifespan in a yeast model (Howitz et al, 2004). It was demonstrated that resveratrol can mimic the benefits of calorie restriction, as seen in figure 8. Interestingly did calorie restriction not further extend lifespan of yeast grown in resveratrol supplemented medium. Resveratrol was shown to extend longevity in worms, flies, fish

and obese mice (Wood et al, 2004; Baur et al, 2006). Another study showed that resveratrol had no effect on longevity of mice fed on a normal diet, but did delay age-related deterioration (Pearson et al, 2008). It is commonly accepted that resveratrol can improve health and prevent age-related diseases but further clarifications are required to verify whether it is a true calorie restriction mimetic.

Rapamycin is another mimetic, able to inhibit the mTORC1 pathway (figure 8). Rapamycin is an antibiotic, immune-suppressing drug, shown to have a longevity benefit (Powers et al, 2006). Rapamycin treatment extends both the median and maximum lifespan, accompanied with a decrease in TORC1 activity, as it was shown in 20-month-old mice (Harrison et al, 2009). More studies have been conducted to confirm the function of rapamycin as a calorie restriction mimetic, but it should also be mentioned that there is evidence showing adverse side-effects of rapamycin, including an increase in the incidence of insulin resistance, cataracts and testicular degeneration (Blattler et al, 2012).

Metformin is a biguanide used as a drug for type-2 diabetes treatment that increases insulin sensitivity and activates AMPK (figure 8). Metformin was identified in a screening assay of drugs showing similar transcriptional profiles to that of caloric restriction in mice (Dhabi et al, 2005). In *C. elegans*, metformin had caloric restriction-related longevity benefits mediated by the activation of AMPK (Onken & Driscoll, 2010).



Beneficial effects were also seen on other aspect of the aging process, especially a decrease in age-related disease incidence. In a non-disease mouse model, metformin improved health span and lifespan (Martin-Montalvo et al, 2013). However as it was seen in Resveratrol and Rapamycin also, not every study showed beneficial effects. The longevity benefit of metformin was not observed in *Drosophila* (Slack et al, 2012).

Figure 8. Potential molecular effectors of resveratrol, metformin and rapamycin for life span regulation (Mouchiroud et al, 2010).

Modulation of the microbiome

The microbiome has an important role in aging. The crucial role of the microbial community inhabiting the gastrointestinal tract is regulating health status and lifespan (Biagi et al, 2016). Health status rely heavily on the microbiome, which play a role in protection against pathogens as well as nutritional status and energy expenditure on the composition of intestinal microbial community (Lakshminarayanan et al, 2014). Several important physiological and metabolic functions of the host organism are influenced by the microbiota, such as driving the maturation of the immune response during early development, and thereby contributing to life-long homeostasis. Control of metabolism, resistance to infection and inflammation, preventing against autoimmunity and cancer, and regulating the brain-gut axis are under control of the gut microbiome (Konturek et al, 2015). The gutmicrobiota is able to influence the risk of many gastrointestinal pathologies, including colorectal cancer, inflammatory bowel disease and irritable bowel syndrome, and some extra-intestinal disorders, including those affecting the liver and respiratory tract (Tojo et al, 2014; Iqbal & Quigley, 2016; Honda & Littman, 2016). Alterations in numbers in microbiota composition seem to play a crucial role in the onset of diseases in the intestinal tract. Changes of composition are affected by both aging and dieting.

Composition of the intestinal microbiota changes substantially with aging, leading to age-related diseases (Lakshminarayanan et al, 2014). These aging-associated alterations in gut physiology have strong effects on the diversity, composition and functional features of the microbiome (Konturek et al, 2015). Age-related changes in gut microbiota composition include a decline in diversity, a decrease in saccharolytic bacteria, an increase in proteolytic bacteria, decreased abundance of core (dominant) species, an increase of subdominant species, an increase of certain Proteobacteria, a reduction of bifidobacterial counts, and a decrease of the ratio Firmicutes to Bacteroides (Bischoff, 2016; Biagi et al, 2016; Pérez Martinez et al, 2014; Rondanelli et al, 2015). To give an example, in vaginally delivered breast-fed infants, the percentage of bifidobacteria numbers of the total colon microbiota decreases throughout life from 90% to less than 5% in adults, and even lower numbers in elderly persons (Riviere et al, 2016). The microbiota of centenarians is less diverse than in adult persons, with decreased levels of Bifidobacterium, Bacteroides and Enterobacteriaceae. The age-related changes in the gut microbiome is an important determinant of age-associated pathological states, such as chronic inflammation, neurodegeneration, cognitive decline, frailty, type 1 and 2 diabetes, non alcoholic fatty liver disease and cardiovascular disease (Rehman, 2012; Friedland, 2015; Magnusson et al, 2015; Meehan et al, 2015; Paun & Danska, 2016; Sanduzzi Zamparelli et al, 2016). Figure 9 displays age-associated changes in human intestinal microbiota composition.

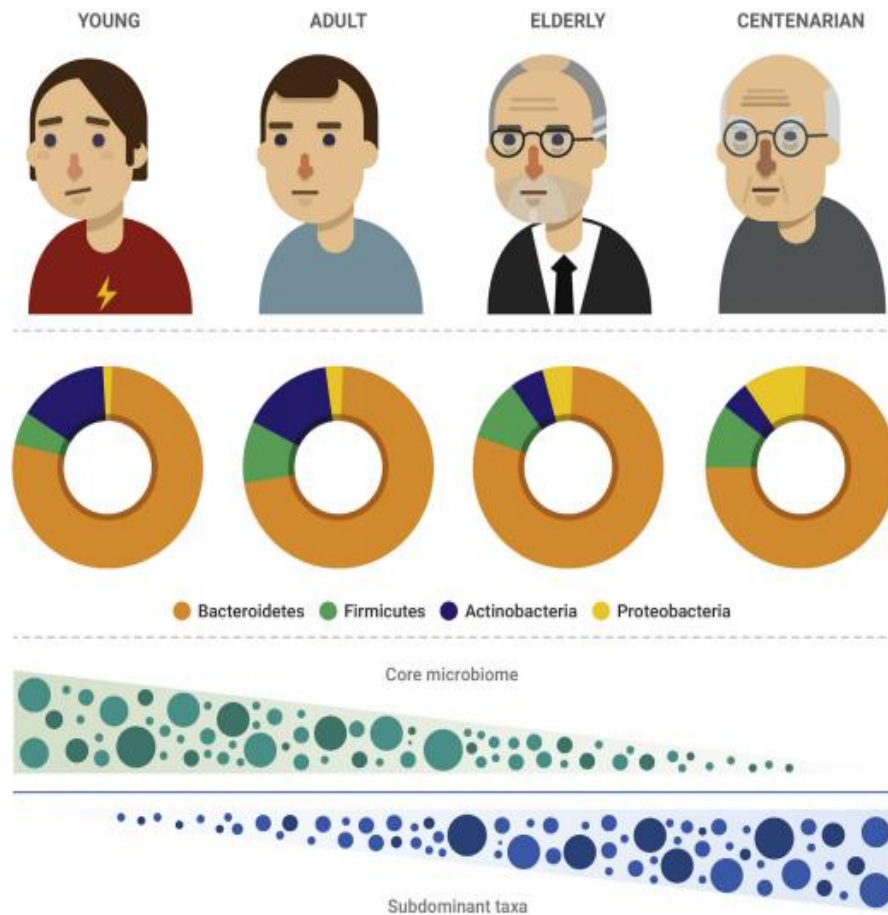


Figure 8. Age-associated changes in human intestinal microbiota composition (Vaiserman et al, 2017).

An interesting find in microbial ecology is the difference between lean and obese subjects. Microbiota of obese individuals is characterized by a decrease in Bacteroides and an increase in Firmicutes, and it is more efficient in harvesting energy from food, than those of normal-weight and lean subjects. Gut microbiota can therefore be a criterion for metabolic health (Graham et al, 2015; Saad et al, 2016; Janssen & Kersten, 2015).

Different studies found an association between the intestinal microbiota composition and weight loss caused by CR. The Firmicutes to Bacteroides ratio was found to be increased in obesity and reduced with weight-loss producing CR-based interventions (Mathur & Barlow, 2015). Research demonstrated that the Firmicutes to Bacteroides ratio decreased significantly in obese individuals receiving a weight-loss dietary intervention (Remely et al, 2015). The weight gain causing bacteria can induce the expression of genes linked to carbohydrate and lipid metabolism, thereby influencing dietary energy harvesting (John & Mullin, 2016). CR-induced life extension in animal models was accompanied by structural modulation of gut microbiota. A life-long low-fat diet significantly altered the overall structure of intestinal microbiota in mice, and CR reduced phylotypes negatively associated with lifespan (Zhang et al, 2013). It is assumed that certain anti-aging interventions may cause specific variations of gut microbial communities causing chronic CR conditions, thus promoting health span and life span (Jonkers, 2016)

Conclusion and future perspectives

The present thesis aims to investigate whether dietary interventions have beneficial effects on extending health span and lifespan, and which molecular mechanisms are involved and whether they are applicable for humans. The first indications positively confirmed this question. Calorie restriction, low-protein high-carbohydrate diets, amino acid restriction, methionine restriction, and time-restricted feeding were all shown to have beneficial effects in regard to lifespan and health span. Most important are improved health span and extended lifespan, with lower onset of aging-related diseases. Some disadvantages were also observed, such as the induction of increased body weight, and adiposity on the low-protein high-carbohydrate diet for a longer period of time. Other studies did not show any effects of the dietary interventions at all. It should be noted that most data came from studies performed on model organisms such as yeast, fruit flies, mice and rats. It is of relevance that pathways and mechanisms can differ greatly between species. The insulin/IGF-1 signal transduction pathway (IIS) is in invertebrates basically one pathway, while the human pathway is more complex and consists of two routes. Moreover is often seen in humans and other mammals, that most of the critical components of the pathway have different forms encoded by different genes and different isoforms encoded by a single gene. The next step is to better understand the precise mechanisms.

Another problem with performing longevity studies on humans is that they take way too long. A lifetime can easily be over a century long, while model organisms have life spans ranging from a few hours to a few months. That is not the only problem to overcome, since it is almost completely impossible for a human to maintain a strict diet throughout their lifetime. It is in humans' nature to eat for pleasure instead of eating for the sole purpose of functionality. The only way to solve this problem is by forcing people, but this is ethically inappropriate. I think that computer simulations can be used in the future, to get better insight in the effects of dietary interventions on humans in the long run.

To overcome to problem of sticking to a strict dietary intervention, while still enjoying the benefits, medicines in the form of dietary restriction mimetics look promising for the future. Resveratrol, Rapamycin and Metformin all seem to have positive effects on both longevity and health span. Further studies should investigate the nutrient sensing pathways better, to develop more specific drugs with less negative side effects. Dietary restriction mimetics could be the Holy Grail in battling aging and aging-related diseases.

Acknowledgements

I would like to thank the European Research Institute for the Biology of Ageing (ERIBA) in Groningen for giving me the opportunity to work on this thesis.

I would like to thank Marco Demaria for allowing me to do my bachelor research in your laboratory. You got me more interested in the process of aging than I already was and were a great role model.

I would like to thank my supervisor, Christine Müller for guiding and supporting me during this bachelor thesis. Your ideas and feedback have been absolutely invaluable.

Finally, I would like to thank my family for giving me constant love, support and motivation through the past couple of years, in particular my mother Anna Boersma.

References

- Akram, M. (2013). A focused review of the role of ketone bodies in health and disease. *J Med Food*, 16(11), 965-967.
- Anisimov, V.N. (2010). Metformin for aging and cancer prevention. *Aging (Albany NY)*, 2(11), 760-774.
- Anselmi, C.V., Malovini, A., Roncarati, R., Novelli, V., Villa, F., Condorelli, G., Bellazzi, R. & Puca, A.A. (2009). Association of the FOXO3A locus with extreme longevity in a southern Italian centenarian study. *Rejuvenation Res*, 12(2), 95-104.
- Apfeld, J., O'Connor, G., McDonagh, T., DiStefano, P.S. & Curtis, R. (2004). The AMP-activated protein kinase AAK-2 links energy levels and insulin-like signals to lifespan in *C. elegans*. *Genes Dev*, 18(24), 3004-3009.
- Apovian, C.M. (2013). The clinical and economic consequences of obesity. *Am J Manag Care*, 19(10), s219-228.
- Aquilano, K., Baldelli, S., Pagliei, B. & Ciriolo, M.R. (2013). Extranuclear Localization of SIRT1 and PGC-1 α : An Insight Into Possible Roles in Diseases Associated With Mitochondrial Dysfunction. *Curr Mol Med*, 13(1), 140-154.
- Argentino, D.P., Dominici, F.P., Al-Regaiey, K., Bonkowski, M.S., Bartke, A. & Turyn, D. (2005) Effects of long-term caloric restriction on early steps of the insulin-signaling system in mouse skeletal muscle. *J Gerontol A Biol Sci Med Sci*, 60(1), 28-34.
- Arriola Apelo, S.I., Neuman, J.C., Baar, E.L., Syed, F.A., Cummings, N.E., Brak, H.K., Pumper, C.P., Kimple, M.E. & Lamming, D.W. (2016). Alternative rapamycin treatment regimes mitigate the impact of rapamycin on glucose homeostasis and the immune system. *Aging Cell*, 15(1), 23-38.
- Asher, G. & Sassone-Corsi, P. (2015). Time for food: the intimate interplay between nutrition, metabolism, and the circadian clock. *Cell*, 161(1), 92-96.
- Baar, E., Carbajal, K.A., Ong, I.M. & Lamming, D.W. (2016). Sex- and tissue-specific changes in mTOR signaling with age in C57BL/6J mice. *Aging Cell*, 15(1), 155-166.
- Bar-Peled, L., Chantranupong, L., Cherniack, A.D., Chen, W.W., Ottina, K.A., Grabiner, B.C., Spear, E.D., Carter, S.L., Meyerson, M. & Sabatini, D.M. (2013). A Tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. *Science*, 340(6136), 1100-1106.

- Baur, J.A., Person, K.J, Price, N.L., Jamieson, H.A., Lerin, C., Kalra, A., Prabhu, V.V., Allard, J.S., Lopez-Lluch, G., Lewis, K., Pistell, P.J., Poosala, S., Becker, K.G., Boss, O., Gwinn, D., Wang, M., Ramaswamy, S., Fishbein, K.W., Spencer, R.G., Lakatta, E.G., Le Couteur, D., Shaw, R.J., Navas, P., Puigserver, P., Ingram, D.K., de Cabo, R. & Sinclair, D.A. (2006). Resveratrol improves health and survival of mice on high-calorie diet. *Nature*, 444(7117), 337-342.
- Belfiora, E., Frasca, F., Pandini, G., Sciacca, L. & Vigneri, R. (2009). Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease. *Endocr Rev*, 30(6), 586-623.
- Besson, A., Salemi, S., Gallati, S., Jenal, A., Horn, R., Mullis, P.S. & Mullis, P.E. (2003). Reduced longevity in untreated patients with isolated growth hormone deficiency. *J Clin Endocrinol Metab*, 88(8), 3664-3667.
- Biagi, E., Nylund, L., Candela, M., Ostan, R., Bucci, L., Pini, E., Nikkila, J., Monti, D., Satokari, R., Franceschi, C., Brigidi, P. & De Vos, W. (2010). Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. *PLoS One*, 5(5), e10667.
- Biagi, E., Franceschi, C., Rampelli, S., Severgnini, M., Ostan, R., Turroni, S., Consolandi, C., Quercia, S., Scurti, M., Monti, D., Capri, M., Brigidi, P. & Candela, M. (2016). Gut microbiota and extreme longevity. *Curr Biol*, 26(11), 1480-1485.
- Bischoff, S.C. (2016). Microbiota and aging. *Curr Opin Clin Nutr Metab Care*, 19(1), 26-30.
- Bjedov, I., Toivonen, J.M., Kerr, F., Slack, C., Jacobson, J., Foley, A. & Partridge, L. (2010). Mechanisms of life span extension by rapamycin in the fruit fly *Drosophila melanogaster*. *Cell Metab*, 11(1), 35-46.
- Blagosklonny, M.V. (2009). TOR-driven aging: speeding car without brakes. *Cell Cycle*, 8(24), 4055-4059.
- Blattler, S.M., Cunningham, J.T., Verdeguer, F., Chim, H., Haas, W., Liu, H., Romanino, K., Ruegg, M.A., Gygi, S.P., Shi, Y. & Puigserver, P. (2012). Yin Yang 1 deficiency in skeletal muscle protects against rapamycin-induced diabetic-like symptoms through activation of insulin/IGF signaling. *Cell Metab*, 15(4), 505-517.
- Bonafè, M., Barbieri, M., Marchegiani, F., Olivieri, F., Ragno, E., Giampieri, C., Mugianesi, E., Centurelli, M., Franceschi, C. & Paolisso, G. (2003). Polymorphic variants of insulin-like growth factor I (IGF-I) receptor and phosphoinositide 3-kinase genes affect IGF-I plasma levels and human longevity: cues for an evolutionarily conserved mechanism of life span control. *J Clin Endocrinol Metab*, 88(7), 3299-3304.
- Cai, D., Dhe-Paganon, S., Melendez, P.A., Lee, J. & Shoelson, S.E. (2003). Two new substrates in insulin signaling IRS5/DOK4 and IRS6/DOK5. *J Biol Chem*, 278(28), 25323-25330.
- Caro, P., Gómez, J., López-Torres, M., Sánchez, I., Naudí, A., Jove, M., Pamplona, R. & Barja, G. (2008). Forty percent and eight percent methionine restriction decrease mitochondrial ROS generation and oxidative stress in rat liver. *Biogerontology*, 9(3), 183-196.
- Caro, P., Gomez, J., Sanchez, I., Naudi, A., Ayala, V., López-Torres, M., Pamplona, R. & Barja, G. (2009). Forty percent methionine restriction decreases mitochondrial oxygen radical production and leak at complex I during forward electron flow and lowers oxidative damage to proteins and mitochondrial DNA in rat kidney and brain mitochondria. *Rejuvenation Res*, 12(6), 421-434.
- Chaix, A., Zarrinpar, A., Miu, P. & Panda, S. (2014). Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. *Cell Metab*, 20(6), 991-1005.
- Chaix, A. & Zarrinpar, A. (2015). The effects of time-restricted feeding on lipid metabolism and adiposity. *Adipocyte*, 4(4), 319-324.
- Chen, C., Liu, Y., Liu, Y. & Zheng, P. (2009). mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells. *Sci Signal*, 2(98), ra75.
- Cho, S.S., Chen, J.A., Sayed, F., Ward, M.E., Gao, F., Nguyen, T.A., Sohn, P.D., Lo, I., Minami, S., Devidze, N., Zhou, Y., Coppola, G. & Gan, L. (2015). SIRT1 deficiency in microglia contributes to cognitive decline in aging and neurodegeneration via epigenetic regulation of IL-1 β . *J Neurosci*, 35(2), 807-818.

- D'Agostino, D.P., Pilla, R., Held, H.E., Landon, C.S., Puchowicz, M., Brunengraber, H., Ari, C., Arnold, P. & Dean, J.B. (2013). Therapeutic ketosis with ketone ester delays central nervous system oxygen toxicity seizures in rats. *Am J Physiol Regul Integr Comp Physiol*, 304(10), R829-836.
- Dai, D.F., Karunadharma, P.P., Chiao, Y.A., Basisty, N., Crispin, D., Hsieh, E.J., Chen, T., Gu, H., Djukovic, D., Raftery, D., Beyer, R.P., MacCoss, M.J. & Rabinovitch, P.S. (2014) Altered proteome turnover and remodeling by short-term caloric restriction or rapamycin rejuvenate the aging heart. *Aging Cell*, 13(3), 529-539.
- Dhabi, J.M., Mote, P.L., Fahy, G.M. & Spindler, S.R. (2005). Identification of potential caloric restriction mimetics by microarray profiling. *Physiol Genomics*, 23(3), 343-350.
- Dijk, D.J., Duffy, J.F. & Czeisler, C.A. (2001). Age-related increase in awakenings: impaired consolidation of nonREM sleep at all circadian phases. *Sleep*, 24(1), 565-577.
- Flachsbart, F., Caliebe, A., Kleindorp, R., Blanché, H., von Eller-Eberstein, H., Nikolaus, S., Schreiber, S. & Nebel, A. (2009). Association of FOXO3A variation with human longevity confirmed in German centenarians. *Proc Natl Acad Sci USA*, 106(8), 2700-2705.
- Friedland, R.P. (2015). Mechanisms of molecular mimicry involving the microbiota in neurodegeneration. *J Alzheimers Dis*, 45(2), 349-362.
- Frye R.A. (2000). Phylogenetic classification of prokaryotic and eukaryotic Sir-2 like proteins. *Biochem Biophys Res Comm*, 273(2), 793-798.
- Gallinetti, J., Harputlugil, E. & Mitchell, J.R. (2013). Amino acid sensing in dietary-restriction-mediated longevity: roles of signal-transducing kinases GCN2 and TOR. *Biochem J*, 449(1), 1-10.
- Goel, N., Stunkard, A.J., Rogers, N.L., Dongen van, H.P.A., Allison, K.C., O'Reardon, J.P., Ahima, R.S., Cummings, D.E., Heo, M. & Dinges, M. (2009). Circadian rhythm profiles in woman with night eating syndrome. *J Biol Rhythms*, 24(4), 85-94.
- Gonzalez, A.A., Kumar, R., Mulligan, J.D., Davis, A.J., Weindruch, R. & Saupe, K.W. (2004). Metabolic adaptations to fasting and chronic caloric restriction in heart, muscle, and liver do not induce changes in AMPK activity. *Am J Physiol Endocrinol Metab*, 287(5), 1032-1037.
- Grabiner, B.C., Nardi, V., Birsov, K., Possemato, R., Shen, K., Sinha, S., Jordan, A., Back, A.H. & Sabatini, D.M. (2014). A diverse array of cancer-associated MTOR mutation are hyperactivating and can predict rapamycin sensitivity. *Cancer Discov*, 4(5), 554-563.
- Graham, C., Mullen, A. & Whelan, K. (2015). Obesity and the gastrointestinal microbiota: a review of associations and mechanisms. *Nutr Rev*, 73(6), 376-385.
- Guertin, D.A., Stevens, D.M., Thoreen, C.C., Burds, A.A., Kalaany, N.Y., Moffat, J., Brown, M., Fitzgerald, K.J. & Sabatini, D.M. (2006). Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKC α , but not S6K1. *Developmental Cell*, 11(6), 859-871.
- Gwinn, D.M., Shackelford, D.B., Egan, D.F., Mihaylova, M.M., Mery, A., Vasquez, D.S., Turk, B.E. & Shaw, R.J. (2008). AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell*, 30(2), 214-226.
- Hall, J.A., Dominy, J.E., Lee, Y. & Puigserver, P. (2013). The sirtuin family's role in aging and age-associated pathologies. *J Clin Invest*, 123(3), 973-979.
- Hansen, M., Taubert, S., Crawford, D., Libina, N., Lee, S.J. & Kenyon, C. (2007). Lifespan extension by condition that inhibit translation in *Caenorhabditis elegans*. *Aging Cell*, 6(1), 95-110.
- Hardie, D.G. (2011). Sensing of energy and nutrients by AMP-activated protein kinase. *Am J Clin Nutr*, 93(4), 891-896.
- Harkness, T.A., Shea, K.A., Legrand, C., Brahmania, M. & Davies, G.F. (2004). A functional analysis reveals dependence on the anaphase-promoting complex for prolonged life span in yeasts. *Genetics*, 168(2), 759-774.
- Harrison, D.E., Strong, R., Sharp, Z.D., Nelson, J.F., Astle, C.M., Flurkey, K., Nadon, N.L., Wilkinson, J.E., Frenkel, K., Carter, C.S., Pahor, M., Javors, M.A., Fernandez, E. & Miller, R.A. (2009). Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature*, 460(7253), 392-395.

- Harrison, S.J., Raubenheimer, D., Simpson, S.J., Godin, J.G. & Betram, S.M. (2014). Towards a synthesis of frameworks in nutritional ecology, interacting effects of protein, carbohydrate and phosphorus on field cricket fitness. *Proc Biol Sci*, 281(1792), 20140539.
- Hastings, M.H., Reddy, A.B. & Maywood, E.S. (2003). A clockwork web: circadian timing in brain periphery, in health and disease. *Nat Rev Neurosci*, 4(8), 649-661.
- Hatori, M., Vollmers, C., Zarrinpar, A., DiTacchio, L., Bushong, E.A., Gill, S., Leblanc, M., Chaix, A., Joens, M., Fitzpatrick, J.A.J., Ellisman, M.H. & Panda, S. (2012). Time-restricted feeding without reducing caloric intake prevents metabolic disease in mice fed a high-fat diet. *Cell Metab*, 15(6), 848-860.
- Heemst van, D. (2010). Insulin, IGF-1 and longevity. *Aging Dis*, 1(2), 147-157.
- Holliday, R. (2006). Food, fertility and longevity. *Biogerontology*, 7(3), 139-141.
- Honda, K. & Littman, D.R. (2016). The microbiota in adaptive immune homeostasis and disease. *Nature*, 535(7610), 75-85.
- Horst van der, A. & Burgering, B.M. (2007). Stressing the role of FoxO proteins in lifespan and disease. *Nat Rev Mol Cell Biol*, 8(6), 440-450.
- Howitz, K.T., Bitterman, K.J., Cohen, H.Y., Lamming, D.W., Lavu, S., Wood, J.G., Zipkin, R.E., Chung, P., Kisielewski, A., Zhang, L.L., Scherer, B. & Sinclair, D.A. (2004). Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature*, 425(6954), 191-196.
- Houtkoper, R.H., Pirinen, E. & Auwerx, J. (2012). Sirtuins as regulators of metabolism and healthspan. *Nat Rev Mol Cell Biol*, 13(4), 225-238.
- Huang, X., Hancock, D.P., Gosby, A.K., McMahon, A.C., Solon, S.M., Le Couteur, D.G., Conigrave, A.D., Raubenheimer, D. & Simpson, S.J. (2013). Effects of dietary protein to carbohydrate balance on energy intake, fat storage, and heat production in mice. *Obesity (Silver Spring)*, 21(1), 85-92.
- Imai, S. & Guarente, L. (2010). Ten years of NAD-dependent SIR2 family deacetylases: implications for metabolic diseases. *Trends Pharmacol Sci*, 31(5), 212-220.
- Inoki, K., Zhu, T. & Guan, K.L. (2003). TSC2 mediates cellular energy response to control cell growth and survival. *Cell*, 115(5), 577-590.
- Iqbal, S. & Quigley, E.M. (2016). Progress in our understanding of the gut microbiome: implications for the clinician. *Curr Gastroenterol Rep*, 18(9), 49.
- Jager, S., Handschin, C., St-Pierre, J. & Spiegelman, B.M. (2007). AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1 α . *Proc Natl Acad Sci USA*, 104(29), 12017-12022.
- Janssen, A.W. & Kersten, S. (2015). The role of the gut microbiota in metabolic health. *FASEB J*, 29(8), 3111-3123.
- Jensen, K., McClure, C., Priest, N.K. & Hunt, J. (2015). Sex-specific effects of protein and carbohydrate intake on reproduction but not lifespan in *Drosophila melanogaster*. *Aging Cell*, 14(4), 605-615.
- John, G.K. & Mullin, G.E. (2016). The gut microbiome and obesity. *Curr Oncol Rep*, 18(7), 45.
- Johnson, S.C., Rabinovitch, P.S. & Kaeberlein. (2013). mTOR is a key modulator of ageing and age-related disease. *Nature*, 493(7432), 338-345.
- Jonkers, D.M. (2016). Microbial perturbations and modulations in conditions associated with malnutrition and malabsorption. *Best Pract Res Clin Gastroenterol*, 30(2), 161-172.
- Kaeberlein, M. & Kennedy, B.K. (2007). Protein translation. *Aging Cell*, 6(6), 731-734.
- Kaeberlein, M. (2008). The ongoing saga of sirtuins and aging. *Cell Metab*, 8(1), 4-5.
- Kalsbeek, A., Foppen, E., Schalijs I., Heijningen van, C., Vliet van der, J., Fliers, E. & Buijs, R.M. (2008). Circadian control of the daily plasma glucose rhythm: an interplay of GABA and glutamate. *PLoS One*, 3(9), e3194.
- Kamdar, B.B., Tergas, A.I., Mateen, F.J., Bhayani, N.H. & Oh, J. (2013). Night-shift work and risk of breast cancer: A systematic review and meta-analysis. *Breast Cancer Res Treat*, 138(1), 291-301.

- Kapahi, P., Zid, B.M., Harper, T., Koslover, D., Sapin, V. & Benzer, S. (2004). Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway. *Curr Biol*, 14(10), 885-890.
- Kennedy, B.K. & Lamming, D.W. (2016). The Mechanistic Target of Rapamycin: The Grand ConducTOR of Metabolism and Aging. *Cell Metab*, 23(6), 990-1003.
- Kenyon, C.J. (2010). The genetics of ageing. *Nature*, 464(7288), 504-512.
- Kondratov, R.V., Kondratova, A.A., Gorbacheva, V.Y., Vukhovanets, O.V. & Antoch, M.P. (2006). Early aging and age-related pathologies in mice deficient in BMAL1, the core component of the circadian clock. *Genes Dev*, 20(14), 1868-1873.
- Konturek, P.C., Haziri, D., Brzozowski, T., Hess, T., Heyman, S., Kwiecien, S., Konturek, S.J. & Koziel, J. (2015). Emerging role of fecal microbiota therapy in the treatment of gastrointestinal and extra-gastrointestinal disease. *J Physiol Pharmacol*, 66(4), 483-491.
- Lakshminarayanan, B., Stanton, C., O'Toole, P.W. & Ross, R.P. (2014). Compositional dynamic of the human intestinal microbiota with aging: implications for health. *J Nutr Health Aging*, 18(9), 773-786.
- Lamming, D.W., Ye, L., Katajisto, P., Goncalves, M.D., Saitoh, M., Stevens, D.M., Davis, J.G., Salmon, A.B., Richardson, A., Ahima, R.S., Guertin, D.A., Sabatini, D.M. & Baur, J.A. (2012). Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. *Science*, 335(6067), 1638-1643.
- Lamming, D.W., Ye, L., Sabatini, D.M. & Baur, J.A. (2013). Rapalogs and mTOR inhibitors as anti-aging therapeutics. *J Clin Invest*, 123(3), 980-989.
- Le Couteur, D.G., Solon-Biet, S., Cogger, V.C., Mitchell, S.J., Senior, A., Cabo de, R., Raubenheimer, D. & Simpson, S.J. (2016). The impact of low-protein high-carbohydrate diets on aging and lifespan. *Cell Mol Life Sci*, 73(6), 1237-1252.
- Lee, B.C., Kaya, A., Ma, S., Kim, G., Garashchenko, M.V., Yim, S.H., Hu, Z. & Harshman, L.G. & Gladyshev, V.N. (2014). *Nat Commun*, 5, 3592.
- Lee, S.H. & Min, K.J. (2013). Caloric restriction and its mimetics. *BMB Rep*, 46(4), 181-187.
- LeSauter, J. & Silver, R. (1998) Output signal of the SCN. *Chronobiol Int*, 15(5), 535-550.
- Li, Y., Xu, W., McBurney, M.W. & Longo, V.D. (2008). SirT1 inhibition reduces IGF-1/IRS-2/Ras/ERK1/2 signaling and protects neurons. *Cell Metab*, 8(1), 38-48.
- Lin, C.H., Lin, C.C., Ting, W.J., Pai, P.Y., Kuo, C.H., Ho, T.J., Kuo, W.W., Chang, C.H., Huang, C.Y. & Lin, W.T. (2014). Resveratrol enhanced FOXO3 phosphorylation via synergetic activation of SIRT1 and PI3K/Akt signaling to improve the effects of exercise in elderly rat hearts. *Age (Dordr)*, 36(5), 9705.
- López-Lluch, G. & Navas, P. (2016). Calorie restriction as an intervention in ageing. *J Physiol*, 594(8), 2043-2060.
- Magnusson, K.R., Hauck, L., Jeffrey, B.M., Elias, V., Humphrey, A., Nath, R., Perrone, A. & Bermudez, L.E. (2015). Relationships between diet-related changes in the gut microbiome and cognitive flexibility. *Neuroscience*, 300, 128-140.
- Mair, W., Morantte, I., Rodrigues, A.P.C., Manning, G., Monitminy, M., Shaw, R.J. & Dillin, A. (2011). Lifespan extension induced by AMPK and calcineurin is mediated by CRTC-1 and CREB. *Nature*, 470(7334), 404-408.
- Malloy, V.L., Perrone, C.E., Mattocks, D.A., Ables, G.P., Caliendo, N.S., Orentreich, D.S. & Orentreich, N. (2013). Methionine restriction prevents the progression of hepatic steatosis in leptin-deficient obese mice. *Metabolism*, 62(11), 1651-1661.
- Manoogian, E.N.C. & Panda S. (2016). Circadian rhythms, time-restricted feeding, and healthy aging. *Ageing Res Rev*, S1568-1637(16), 30301-30304.
- Martin-Montalvo, A., Mercken, E.M., Mitchell, S.J., Palacois, H.H., Mote, P.L., Scheibye-Knudsen, M., Gomes, A.P., Ward, T.M., Minor, R.K., Blouin, M.J., Schwab, M., Pollak, M., Zhang, Y., Yu, Y., Becker, K.G., Bohr, V.A., Ingram, D.K., Sinclair, D.A., Wolf, N.S., Spindler, S.R., Bernier, M. & de Cabo, R. (2013). Metformin improves healthspan and lifespan in mice. *Nat Commun*, 4, 2192.
- Mathur, R. & Barlow, G.M. (2015). Obesity and the microbiome. *Expert Rev Gastroenterol Hepatol*, 9(8), 1087-1099.

- McCay, C.M., Crowel, M.F. & Maynard, L.A. (1935). The Effect of Retarded Growth Upon the Length of Life Span and Upon the Ultimate Body Size. *J Nutr*, 10(1), 63-79.
- Meehan, C.J., Langille, M.G. & Beiko, R.G. (2015). Frailty and the microbiome. *Interdiscip Top Gerontol Geriatr*, 41, 54-65.
- Michan, S. (2014). Calorie restriction and NAD⁺/sirtuin counteract the hallmarks of aging. *Front Biosci (Landmark Ed)*, 19, 1300-1319.
- Miller, B.F., Robinson, M.M., Bruss, M.D., Hellerstein, M. & Hamilton, K.L. (2012). A comprehensive assessment of mitochondrial protein synthesis and cellular proliferation with age and caloric restriction. *Aging Cell*, 11(1), 150-161.
- Minor, R.K., Allard, J.S., Younts, C.M., Ward, T.M. & Cabo de, R. (2010). Dietary interventions to extend life span and health span based on calorie restriction. *J Gerontol A Biol Sci Med Sci*, 65(7), 695-703.
- Mouchiroud, L., Molin, L., Dalliere, N. & Solari, F. (2010). Life span extension by resveratrol, rapamycin, and metformin: The promise of dietary restriction mimetics for an healthy aging. *BioFactors*, 36(5), 377-382.
- Okazaki, M., Iwasaki, Y., Nishiyama, M., Taguchi, T., Tsugita, M., Nakayama, S., Kambayashi, M., Hashimoto, K. & Terada, Y. (2015). PPARbeta/delta regulates the human SIRT1 gene transcription via Sp1. *Endocr J*, 57(5), 403-413.
- Onken, B. & Driscoll, M. (2010). Metformin induces a dietary restriction-like state and the oxidative stress response to extend *C. elegans* Healthspan via AMPK, LKB1, and SKN-1. *PLoS One*, 5(1), e8758.
- Orentreich, N., Matias, J.R., DeFelice, A. & Zimmerman, J.A. (1993). Low methionine ingestion by rats extends life span. *J Nutr*, 123(2), 269-274.
- Pan, K.Z., Palter, J.E., Rogers, A.N., Olsen, A., Chen, D., Lithgow, G.J. & Kapahi, P. (2007). Inhibition of mRNA translation extends lifespan in *Caenorhabditis elegans*. *Aging Cell*, 6(1), 111-119.
- Paun, A. & Danska, J.S. (2016). Modulation of type 1 and type 2 diabetes risk by the intestinal microbiome. *Pediatr Diabetes*, 17(7), 469-477.
- Pearson, K.J., Baur, J.A., Lewis, K.N., Peshkin, L., Price, N.L., Labinsky, N., Swindell, W.R., Kamara, D., Minor R.K., Perez, E., Jamieson, H.A., Zhang, Y., Dunn, S.R., Sharma, K., Pleshko, N., Woollett, L.A., Csiszar, A., Ikeno, Y., Le Couteur, D., Elliot, P.J., Becker, K.G., Navas, P., Ingram, D.K., Wolf, N.S., Ungyari, Z., Sinclair, D.A. & de cabo, R. (2008). Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. *Cell Metab*, 8(2), 157-168.
- Pendergast, J.S., Branecky, K.L., Yang, W., Ellacott, K.L.J., Niswender, K.D. & Yamazaki, S. (2013). High-fat diet acutely affects circadian organization and eating behavior. *Eur J Neurosci*, 37(8), 1350-1356.
- Pérez Martínez, G., Bauerl, C. & Collado, M.C. (2014). Understanding gut microbiota in elderly's health will enable intervention through probiotics. *Benef Microbes*, 5(3), 235-246.
- Piper, M.D. & Bartke, A. (2008). Diet and aging. *Cell Metab*, 8(2), 99-104.
- Powers, R.W., Kaeberlein, M., Caldwell, S.D., Kennedy, B.K. & Fields, S. (2006). Extension of chronological life span in yeast by decreased TOR pathway signaling. *Genes Dev*, 20(2), 174-184.
- Proper, K.I., Langenberg van de, D., Rodenburg, W., Vermeulen, R.C., Beek van der, A.J., Steeg van, H. & Kerkhof van, L.W. (2016). The relationship between shift work and metabolic risk factors: a systematic review of longitudinal studies. *Am J Prev Med*, 50(5), e147-157.
- Purushotham, A., Schug, T., Xu, Q., Surapureddi, S., Guo, X. & Li, X. (2009). Hepatocyte-specific deletion of SIRT1 alters fatty acids metabolism and results in hepatic steatosis and inflammation. *Cell Metab*, 9(4), 327-338.
- Ramis, M.R., Esteban, S., Miralles, A., Tax, D.X. & Reiter, R.J. (2015). Caloric restriction, resveratrol and melatonin: Role of SIRT1 and implications for aging and related-diseases. *Mech Ageing Dev*, 146-148, 28-41.
- Rehman, T. (2012). Role of the gut microbiota in age-related chronic inflammation. *Endocr Metab Immune Disord Drug Targets*, 12(4), 361-367.

- Remely, M., Tesar, I., Hippe, B., Gnauer, S., Rust, P. & Haslberger, A.G. (2015). Gut microbiota composition correlates with changes in body fat content due to weight loss. *Benef Microbes*, 6(41), 431-439.
- Rievriere, A., Selak, M., Lantin, D., Leroy, F. & De Vuyst, L. (2016). Bifidobacteria and butyrate-producing colon bacteria: importance and strategies for their stimulation in the human gut. *Front Microbiol*, 7, 979.
- Robida-Stubbs, S., Glover-Cutter, K., Lamming, D.W., Mizunuma, M., Narasimhan, S.D., Neumann-Haefelin, E., Sabatini, D.M. & Blackwell, T.K. (2012). TOR signaling and rapamycin influence longevity by regulating SKN-1/Nrf and DAF-16/FoxO. *Cell Metab*, 15(5), 713-724.
- Rondanelli, M., Giacosa, A., Faliva, M.A., Perna, S., Allieri, F., & Castellazzi, A.M. (2015). Review on microbiota and effectiveness of probiotics use in older. *World J Clin*, 3(2), 156-162.
- Saad, M.J., Santos, A. & Prada, P.O. (2016). Linking gut microbiota and inflammation to obesity and insulin resistance. *Physiology (Bethesda)*, 31(4), 283-293.
- Sadacca, L.A., Lamia, K.A., DeLemos, A.S., Blum, B. & Weitz, C.J. (2011). An intrinsic circadian clock of the pancreas is required for normal insulin release and glucose homeostasis in mice. *Diabetologia*, 54(1), 120-124.
- Sanchez-Roman, I., Gomez, A. & Gomez, J. (2011). Forty percent methionine restriction lowers DNAmethylations, complex iROS generation, and oxidative damage to mtDNA and mitochondrial proteins in rat heart. *J Bioenerg Biomembr*, 43(6), 699-708.
- Sanduzzi Zamparelli, M., Compare, D., Coccoli, P., Rocco, A., Nardone, O.M., Marrone, G., Gasbarrini, A., Grieco, A., Nardone, G. & Miele, L. (2016). The metabolic role of gut microbiota in the development of nonalcoholic fatty liver disease and cardiovascular disease. *Int J Mol Sci*, 17(8), E1225.
- Sanz, A., Caro, P., Ayala, V., Portero-Otin, M., Pamplona, R. & Barja, G. (2006). Methionine restriction decreases mitochondrial oxygen radical generation and leak as well as oxidative damage to mitochondrial DNA and proteins. *FASEB J*, 20(8), 1064-1073.
- Selman, C., Tullet, J.M., Wieser, D., Irvine, E., Lingard, S.J., Choudhury, A.I., Claret, M., Al-Qassab, H., Carmignac, D., Ramadani, F., Woods, A., Robinson, I.C., Schuster, E., Batterham, R.L., Kozma, S.C., Thomas, G., Carling, D., Okkenhaug, K., Thornton, J.M., Partridge, L., Gems, D. & Withers, D.J. (2009). Ribosomal protein S6 kinase 1 signaling regulates mammalian life span. *Science*, 236(5949), 140-144.
- Sengupta, S., Peterson, T.R. & Sabatini, D.M. (2010). Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. *Mol Cell*, 40(2), 310-322.
- Sharp, Z.D. (2011). Aging and TOR: interwoven in the fabric of life. *Cell Mol Life Sci*, 68(4), 587-598.
- Sherman, H., Genzer, Y., Cohen, R., Chapnik, N., Madar, Z. & Froy, O. (2012). Timed high-fat diet resets circadian metabolism and prevent obesity. *FASEB J*, 26(8), 3493-3502.
- Simpson, S.J. & Raubenheimer, D. (2005). Obesity: the protein leverage hypothesis. *Obes Rev*, 6(2), 133-142.
- Simpson, S.J., Le Couteur, D.G. & Raubenheimer, D. (2015). Putting the balance back in diet. *Cell*, 161(1), 18-23.
- Slack, C., Foley A. & Patridge, L. (2012). Activation of AMPK by the putative dietary restriction mimetic metformin is insufficient to extend lifespan in *Drosophila*. *PloS One*, 7(10), e47699.
- So, A.Y.-L., Bernal, T.U., Pillsbury M.L., Yamamoto, K.R. & Feldman, B.J. (2009). Glucocorticoid regulation of the circadian clock modulates glucose homeostasis. *Proc Natl Acad Sci USA*, 106(41), 17582-17587.
- Solon-Biet, S.M., McMahon, A.C., Ballard, J.W., Ruohonen, K., Wu, L.E., Cogger, V.C., Warren, A., Huang, X., Pichaud, N., Melvin, R.G., Gokarn, R., Khalil, M., Turner, M., Cooney, G.J., Sinclair, D.A., Raubenheimer, D., Le Couteur, D.G. & Simpson, S.J. (2014). The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice. *Cell Metab*, 19(3), 418-430.

- Solon-Biet, S.M., Mitchell, S.J., Cabo de, R., Raubenheimer, D., Le Couteur, D.G. & Simpson, S.J. (2015). Macronutrients and caloric intake in health and longevity. *J Endocrinol*, 226(1), R17-28.
- Solon-Biet, S.M., Mitchell, S.J., Coogan, S.C., Cogger, S.C.P., Cogger, V.C., Gokarn, R., McMahon, A.C., Raubenheimer, D., Cabo de, R., Simpson, S.J. & Le Couteur, D.G. (2015). Dietary protein to carbohydrate ratio and caloric restriction: comparing metabolic outcomes in mice. *Cell Rep*, 11(10), 1529, 1534.
- Sorensen, A., Mayntz, D., Raubenheimer, D. & Simpson, S.J. (2008). Protein-leverage in mice: the geometry of macronutrient balancing and consequences for fat deposition. *Obesity (Silver Spring)*, 16(3), 556-571.
- Spilman, P., Podlitskaya, N., Hart, M.J., Debnath, J., Gorostiza, O., Bredesen, D., Richardson, A., Strong, R. & Galvan, V. (2010). Inhibition of mTOR by rapamycin abolishes cognitive deficits and reduces amyloid-beta levels in a mouse model of Alzheimer's disease. *PLoS One*, 5(4), e9979.
- Stock, M.J. (1999). Gluttony and thermogenesis revisited. *Int J Obes Relat Metab Disord*, 23(11), 1105-1117.
- Sturm, R. & Hattori, A. (2013). Morbid obesity rates continue to rise rapidly in the United States. *Int J Obes (Lond)*, 37(6), 889-891.
- Suh, Y., Atmon, G., Cho, M.O., Hwang, D., Liu, B., Leahy, D.J., Barzilai, N. & Cohen, P. (2008). Functionally significant insulin-like growth factor I receptor mutations in centenarians. *Proc Natl Acad Sci USA*, 105(9), 3438-3442.
- Sun, L., Sadighi Akha, A.A., Miller, R.A. & Harper, J.M. (2009). Life-span extension in mice by preweaning food restriction and by methionine restriction in middle age. *J Gerontol A Biol Med Sci*, 64(7), 711-722.
- Sweeney G. & Song, J. (2016). The association between PGC-1 α and Alzheimer's disease. *Anat Cell Biol*, 49(1), 1-6.
- Takano, A., Usui, I., Haruta, T., Kawahara, J., Uno, T., Iwata, M. & Kobayashi, M. (2001). Mammalian target of rapamycin pathway regulates insulin via subcellular redistribution of insulin receptor substrate 1 and integrates nutritional signals and metabolic signals of insulin. *Mol Cell Biol*, 21(15), 5050-5062.
- Tallóczy, Z., Jiang, W., Virgin, H.W., Leib, D.A., Scheuner, D., Kaufman, R.J., Eskelinen, E.L. & Levine, B. (2002). Regulation of starvation- and virus-induced autophagy by the eIF2 α kinase signaling pathway. *Proc Natl Acad Sci USA*, 99(1), 190-195.
- Taniguchi, C.M., Emanuelli, B. & Kahn, C.R. (2006). Critical nodes in signaling pathways: insights into insulin action. *Nat Rev Mol Cell Biol*, 7(2), 85-96.
- Testa, G., Biasi, F., Poli, G. & Chiarpotto, E. (2014). Calorie restriction and dietary restriction mimetics: a strategy for improving healthy aging and longevity. *Curr Pharm Des*, 20(18), 2950-2977.
- To, K., Yamaza, H., Kamatsu, T., Hayashida, T., Hayashi, H., Toyama, H., Chiba, T., Higama, Y. & Shimokawa, I. (2007). Down-regulation of AMP-activated protein kinase by calorie restriction in rat liver. *Exp Gerontol*, 42(11), 1063-1071.
- Tohyama D. & Yamaguchi, A. (2010). A critical role of SNF1A/dAMPK α (Drosophila AMP-activated protein kinase α) in muscle on longevity and stress resistance in *Drosophila melanogaster*. *Biochem Biophys Res Commun*, 394(1), 112-118.
- Tojo, R., Suarez, A., Clemente, M.G., de los Reyes-Gavilan, C.G., Margolles, A., Gueimonde, M. & Ruas-Madiedo, P. (2014). Intestinal microbiota in health and disease: role of bifidobacteria in gut homeostasis. *World J Gastroenterol*, 20(41), 15163-15176.
- Torra, I.P., Tsibulsky, V., Delaunay, F., Saladian, R., Laudet, V., Fruchart, J.C., Kosykh, V. & Staels, B. (2000). Circadian and glucocorticoid regulation of Rev-erb α expression in liver. *Endocrinology*, 141(10), 3799-3806.
- Tran, D., Bergholz, J., Zhang, H., He, H., Wang, Y., Zhang, Y., Li, Q., Kirkland, J.L. & Xiao, Z.X. (2014). Insulin-Like Growth Factor-1 Regulates the SIRT1-p53 Pathway in cellular Senescence. *Aging Cell*, 13(4), 669-678.

- Troen, A.M., French, E.E., Roberts, J.F., Selhub, J., Ordovas, J.M., Pamell, L.D. & Lai, C.Q. (2007). Lifespan modification by glucose and methionine in *Drosophila melanogaster* fed a chemically defined diet. *Age(Dordr)*, 29(1), 29-39.
- Vaiserman, A.M., Koliada, A.K. & Marotta, F. (2017). Gut microbiota: A player in aging and a target for anti-aging intervention. *Ageing Res Rev*, 35, 36-45.
- Vellai, T., Takacs-Vellai, K., Zhang, Y., Kovacs, A.L., Orosz, L. & Müller, F. (2003). Genetics: influence of TOR kinase on lifespan in *C. elegans*. *Nature*, 426(6967), 620.
- Vézina, C., Kudelski, A. & Sehgal, S.N. (1975). Rapamycin (AY-22,989), a new antifungal antibiotic. 1. Taxonomy of the producing streptomycete and isolation of the active principle. *J Antibiot (Tokyo)*, 28(10), 721-726.
- Vyas, M.V., Garg, A.X., Iansavichus, A.V., Costella, J., Donner, A., Laugsand, L.E., Janszky, I., Mrkobrada, M., Parraga, G. & Hackham, D.G. (2012) Shift work and vascular events:systematic review and meta-analysis. *BMJ*, 345, e4800.
- Weiss, E.P., Racette, S.B., Villareal, D.T., Fontana, L., Steger-May, K., Schechtman, K.B., Klein, S., Holloszy, J.O. & Washington University School of Medicine CALERIE Group. (2006). Improvements in glucose tolerance and insulin action induced by increasing energy expenditure or decreasing energy intake: a randomized controlled trial. *Am J Clin Nutr*, 84(5), 1033-1042.
- Welsh, D.K., Takahashi, J.S. & Kay, S.A. (2010). Suprachiasmatic nucleus: cell autonomy and network properties. *Annu Rev Physiol*, 72, 551-577.
- Wilkinson, J.E., Burmeister, L., Brooks, S.V., Chan, C.C., Friedline, S., Harrison, D.E., Hejtmanick, J.F., Nadon, N., Strong, R., Wood, L.K., Woodward, M.A. & Miller, R.A. (2012). Rapamycin slows aging in mice. *Ageing Cell*, 11(4), 675-682.
- Willcox, B.J., Donclon, T.A., He, Q., Chen, R., Groves, J.S., Yano, K., Masaki, K.H., Willcox, D.C., Rodriguez, B. & Curb, J.D. (2008). FOXO3A genotype is strongly associated with human longevity. *Proc Natl Acad Sci USA*, 105(37), 13987-13992.
- Wood, J.G., Rogina, B., Lavu, S., Howitz, K., Helfand, S.L., Tatar, M. & Sinclair, D. (2004). Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature*, 470(7000), 686-689.
- Yamamoto, T., Nakahata, Y., Tanaka, M., Yoshida, M., Soma, H., Shinohara, K., Yasuda, A., Mamine, T. & Takumi, T. (2005). Acute physical stress elevates mouse Period1 mRNA expression in mouse peripheral tissues via a glucocorticoid-responsive element. *J Biol Chem*, 280(51), 42036-42043.
- Yao, H., Chung, S., Hwang, J.W., Rajendrasozhan, S., Sundar, I.K., Dean, D.A., McBurney, M.W., Guarente, L., Gu, W., Rönty M., Kinnula, V.L. & Rahman, I. (2012). SIRT1 protects against emphysema via FOXO3-mediated reduction of premature senescence in mice. *J Clin Invest*, 122(6), 2032-2045.
- Yuan, Y., Cruzat, V.F., Newsholme, P., Cheng, J., Chen Y. & Lu, Y. (2016). Regulation of SIRT1 in aging: Roles in mitochondrial function and biogenesis. *Mech Ageing Dev*, 155, 10-21.
- Zhang, C., Li, S., Yang, L., Huang, P., Li, W., Wang, S., Zhao, G., Zhang, M., Pang, X., Yan, Z., Liu, Y. & Zhao, L. (2013). Structural modulation of gut microbiota in life-long calorie-restricted mice. *Nat Commun*, 4, 2163.