The effect of the dietary intervention's calorie restriction and low protein/high carbohydrate diet on lifespan

A literature review of the current knowledge.

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Preface

As long as I can remember, I have been fascinated with nutrition and research. From presenting news articles about obesity in primary school to intermitted fasting challenges in college with friends. Over the past decades, the diabetic and obese Western society increased significantly, and more is known about the effects of nutrition on age-related diseases including dementia, Alzheimers and Parkinson's Disease. Therefore, it is deeply interesting to explore the balance of food and aging. As such, I have made it my mission to explore and help to improve the field of healthy ageing in context of nutrition.
With this thesis on the topic of dietary interventions increasing lifespan, I would like to educate myself and others on the subject to become more aware of the urge of an optimal diet, preventing oneself from unnecessary suffering. A better understanding of the relation between dietary interventions and lifespan is essential for the survival of our species.

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Abstract

Currently, the maximal lifespan in humans is higher than ever before and, even more persistently, it is prognosed to continue to increase in the coming decades. Unfortunately, this prolonged lifetime does not come without a cost. The Western world suffers from chronic diseases as, for example, obesity, diabetes type 2, cardiovascular disease, and fatty liver. Since it was suggested almost a century ago that dietary interventions might prevent or, at least, reduce age-related complications, a variety of studies has been conducted to compose the most effective diet. Best known is the dietary intervention of calorie restriction of 30-50% without malnutrition to reduce to onset of age-related diseases. However, more recently, it has been demonstrated that the balance of a low protein/ high carbohydrate diet prolongs health and lifespan even better. As many studies have shown contradictory results in a search for the best dietary intervention, the aim of this extensive scientific review is to provide and clarify the current understandings of both calorie restriction and a low protein/high carbohydrate diet. In addition, it attempts to understand the underlying mechanisms of aging to base a conclusion of which of the two diets increases lifespan best.

Table of contents

1. Introduction

As of today, the human species is living longer than ever before, and it is predicted that this increase in lifespan will continue in the coming decades. There is a probability of over 50% that by 2030, the national female life expectancy will break the 90-year barrier[1] (*Figure 1*). However, continued increased longevity is not without complications. Specifically, prolonged lifespan is associated with well-established chronic diseases as for example, obesity, diabetes type 2, cardiovascular disease, and fatty liver. It has been known for over a century that dietary intervention might prevent or, at least, reduce age-related complications[2].

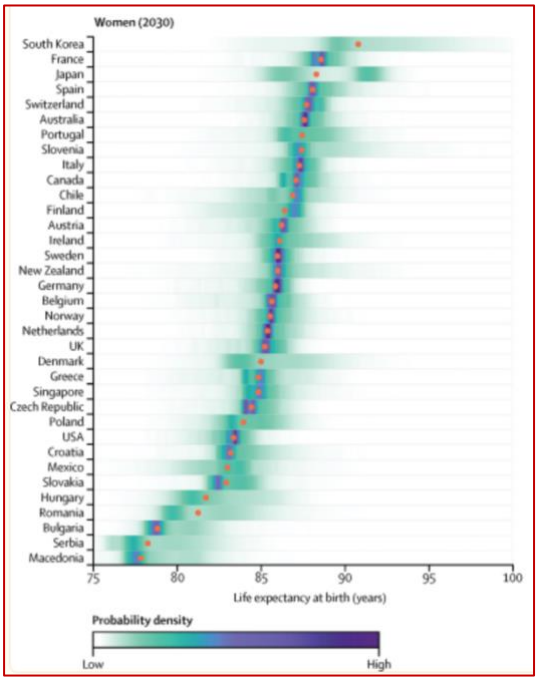

Figure 1. (Taken from Kontis et al. (2017). Life expectancy at birth in years in 2030 per country[1].

Best known is the dietary intervention of a calorie restriction (CR) of 30-50% which shows profound anti-aging effects and reduces the onset of age-associated disease[3–5]. It has been shown that food restriction without malnutrition improves metabolic health and reduces the risk of developing diabetes type 2, cancer, and cardiovascular disease[6,7]. The mechanisms underlying this are gradually being unraveled. Initially, it was proposed that CR slowed down development, however later, it was confirmed that CR is associated with reduced oxidative stress and decreased production of free radicals. CR operates along multiple pathways including the insulin/IGF-1 pathway, the sirtuin pathway and the mTOR pathway[8]. Unfortunately, the dietary intervention of CR is, although very beneficial, difficult to maintain voluntarily by humans, due to a constant appetite[9].

Recently, evidence was found that the balance of macronutrients taken in has a sincere effect on the healthspan. Lifespan was maximized in invertebrates, ad libitum fed mice and *Drosophila* with a low protein, high carbohydrate (LPHC) diet[10,11]. It has been shown that LPHC diets increase cardiometabolic health and influence the immune system positively[11]. Also, telomere shortage, which has been proposed as a biomarker for aging, has been shown to reduce as an effect of a LPHC diet. Moreover, mice on a LPHC diet also showed a prolonged lifespan[12]. Interestingly, all the studies mentioned focused on the balance of macronutrients found that CR did not increase lifespan[11].

Although a LPHC diet might have more profound effects than CR, LPHC diets are associated with overconsumption[13,14]. The 'protein leverage hypothesis', which proposes that a dominant appetite for protein in a LPHC diet drives extra food intake, was proven and, it was shown that this enhances the risk for potential weight gain[13]. The detrimental consequences of overconsumption are well known and lead to obesity, diabetes type 2 and fatty liver[15]. In humans the results of a LPHC diet are less well established, however, LPHC diets are associated with obesity and fatty liver[16,17].

Numerous studies provide conflicting results on the effects of CR and LPHC on aging. Therefore, this report attempts to explore, evaluate, and clarify the current understandings of both dietary interventions, namely CR or LPHC, and which of the two diets increases lifespan best. Even though, CR has been known for long to have profound health and anti-aging effects, it is hypothesized that a well determined LPHC diet increases health- and lifespan even better.

2. Calorie restriction (CR)

2.1 Definitions

Calorie restriction (CR) is a nutritional intervention of reduced energy intake by 30-50% without the lack of essential nutrients. Throughout literature the term CR and dietary restriction (DR) are often used interchangeably, however, this is not correct. CR is an example of DR while, on the other hand, DR, next to CR, also includes reduction of specific macronutrients, changes in the ratio of macronutrients or other dietary interventions as for instance a ketone diet or intermitted fasting[18]. In this piece the effect of CR on healthy ageing and a maximal lifespan is examined. Ageing is defined as an accumulation of time related progressive physiological changes in an organism and a decline of biological functions necessary for survival and fertility of the organism[19]. Healthy aging or healthspan is referred to by means of aging as a process of maintaining functional ability to enable wellbeing in older age[20]. Healthspan ends in the onset of age-related diseases and is followed by the morbidity span. Lifespan is the period of time between birth and death of an organism, whereas maximal lifespan refers to the natural limit of human life[21]. It includes the healthspan and the morbidity span of an individual[22]. Longevity and life expectancy are used as synonyms and are generally best considered meaning 'typical length of life'[21]. The quality of life is the degree to which an individual is healthy, comfortable and able to participate and enjoy events in life[21]. After clarification of the terminology used, we will now focus on the current status of knowledge of CR in relation to healthy aging.

2.2 Early contributions

More than a century ago, it was shown by Osborne (1917) that a reduction in caloric intake of rats increased lifespan. At first, this research did not receive much attention as the study was poorly conducted, and, moreover, the opposite result was shown by Robertson and Ray (1920) who reported that growth rate and lifespan are positively correlated in mice. However, approximately 15 years later, McCay (1935) showed, now in a well conducted study, that CR increased maximal lifespan of rats two-fold compared to rats on a normal diet. Since that moment CR has been under investigation in relation to the length of life in many organisms including rats, mice, yeast and invertebrates[23]. Conflicting results have been presented as some studies showed that a part of the life extension resulting from a reduced food intakes was due to decreased protein intake[24], while others found that restricting the caloric intake by 40% resulted in the same magnitude of life extension, whether protein intake was restricted or not. Masoro (2005) concluded that the results of the study by Yu et al. (1985) are probably related to a misinterpretation in kidney failure as a major cause of death. Namely, in contrast, kidney failure is not a significant cause of death in rats on energy restricted diets. Therefore, Masoro et al. (1989) strongly supported the notion that an increase in lifespan and a reduction in the onset of chronical diseases is a result of CR, rather than protein intake[23]. Since, many studies have concluded the same in all different animals including budding yeast, *C. elegans*, and rodents[25,26]

2.3 Effects on lifespan

Long term CR without malnutrition is a robust intervention known to increase maximal lifespan and healthspan[27,28]. CR has shown to prevent a wide range of chronic non communicable diseases (CNCD) as for example cancer, atherosclerosis, diabetes, cardiovascular diseases, kidney diseases, autoimmune and neurodegenerative disease[18,27]. Also, CR has been reported to enhance DNA repair mechanisms, promote autophagy and apoptosis and reduce oxidative stress[18,27]. Furthermore, CR reduces fasting insulin levels, several growth factors, and cytokines[18,27]. The

molecular mechanisms underlying the beneficial effects of CR target the mTOR pathway, the insulin/IGF-I pathway and the sirtuin pathway[8].

2.3.2 mTOR pathway

To start off with the Mammalian Target for Rapamycin (mTOR) which is a serine-threonine kinase protein complex, and it is found in various cell types throughout the body. The mTOR complex integrates signals from multiple pathways and regulates growth factors, hormones, cytokines and oxidative stress. mTOR has two major protein complexes: mTORC1, which influences numerous cellular functions including protein synthesis and autophagy; and MTORC2, which influences some of the activities of insulin and IGF-1 receptors and the cytoskeleton[8]. Importantly, hyperactivation of mTOR results in inhibition of autophagy and proteolysis and an accumulation of cell debris[8,29]. Therefore, the mTOR pathway is known to contribute to the process of aging. The mTOR pathway is inhibited by AMP-activated protein kinase (AMPK). AMPK is activated upon metabolic stress, which, for instance, could be created by CR. AMPK is a heterotrimeric Ser/Thr kinase composed of α, β and γ subunit[30]. It stimulates the generation of ATP and inhibits ATP consuming processes that are not required for immediate cell survival. Upon energy stress, AMP and ADP concentrations increase and bind to the AMPKγ subunit[31]. This promotes a conformational change that renders AMPK a poorer substrate for dephosphorylation. Then, the increased phosphorylation levels of the threonine residue, resulting in the full activation of the enzyme. Activated AMPK inhibits the kinase activity of mTOR which results in an increase of autophagy[32]. Autophagy is a survival mechanism that plays a housekeeping role in removing misfolded protein aggregates, also, it is important in clearing damaged cell organelles and it eliminates intracellular pathogens[33]. Therefore, the dietary intervention CR has a beneficial effect on the inhibition of the mTOR pathway resulting in increasing lifespan[29].

2.3.1 Insulin/IGF-I pathway

Secondly, it has been reported that the insulin/IGF-1 signaling pathway controls the lifespan in *C. elegans* and mammalian species[34,35]. These studies concluded that deficiencies in the functioning of the insulin/IGF-1 pathway can extend lifespan, whereas an increased activity of the pathway promotes the aging process[36]. The main functions of the insulin/IGF-1 signaling pathway are the regulation of protein synthesis and energy metabolism and the proliferation and differentiation of insulin/IGF-1 responsive cells[36]. Also, the pathway plays an important role in anti-inflammatory and immune responses via the activation of STAT3. On the other hand, the insulin/IGF pathway controls and upregulates the activity of several longevity genes such as mTOR and forkhead box O (FOXO) signaling[37,38]. mTOR activates the Pi3K>AKT pathway, downstream of the insulin/IGF-1 pathway and inhibits autophagy, thereby, accelerating the aging process[37]. The insulin/IGF pathway is activated by insulin and insulin growth factor (IGF). During CR, the insulin levels decrease because of limited glucose intake, and this results in inhibition of the insulin/IGF-1 pathway and eventually in inhibition of mTOR[39]. Thereby, the inhibition of autophagy is decreased, which is associated with increased longevity.

2.3.3 Sirtuin pathway

Lastly, the sirtuin pathway is affected by CR. Sirtuins are nicotinamide dinucleotide (NAD+)-dependent deacetylases and are known to regulate diverse cellular processes including DNA repair, insulin sensitivity and inflammation[40,41]. It has been shown that an extra copy of SIR2, which is a member of the Sirtuin family in budding yeast, extended the lifespan by 30%[42]. SIRT1 in humans showed the highest sequence homology to yeast SIR2. SIRT1 in humans is mainly found in the nucleus or in a shuttle between the cytoplasm and the nucleus and regulates the balance

between bone formation and absorption[43]. It controls the differentiation of mesenchymal stem cells and bone marrow-derived macrophages, thereby increasing osteogenesis. CR upregulates SIRT1 expression directly and indirectly via upregulation of AMPK and an increased NAD+/NADH ratio. It has been reported that a reduction of SIRT1 resulted in increased DNA damage and in an aged phenotype, whereas an overexpression of SIRT1 delayed senescence of bone marrow stem cells[44]. Cellular senescence is a central hallmark of aging and is a physiological phenotype aimed at permanent cell cycle arrest induced by cellular stresses[45-47]. Sirtuins are an essential factor in delaying cellular senescence and, thereby, extending lifespan. Sirtuins also modulate the senescence of stem cells and are required for the maintenance of stem cell self-renewal[48]. Thus, an upregulation of sirtuin activity increases lifespan as a consequence of CR.

In summary, the mTOR pathway, the insulin/IGF-1 pathway and the sirtuin pathways are all directly and/or indirectly influenced by CR in such a way that it has a positive effect on the lifespan (*Figure 2*).

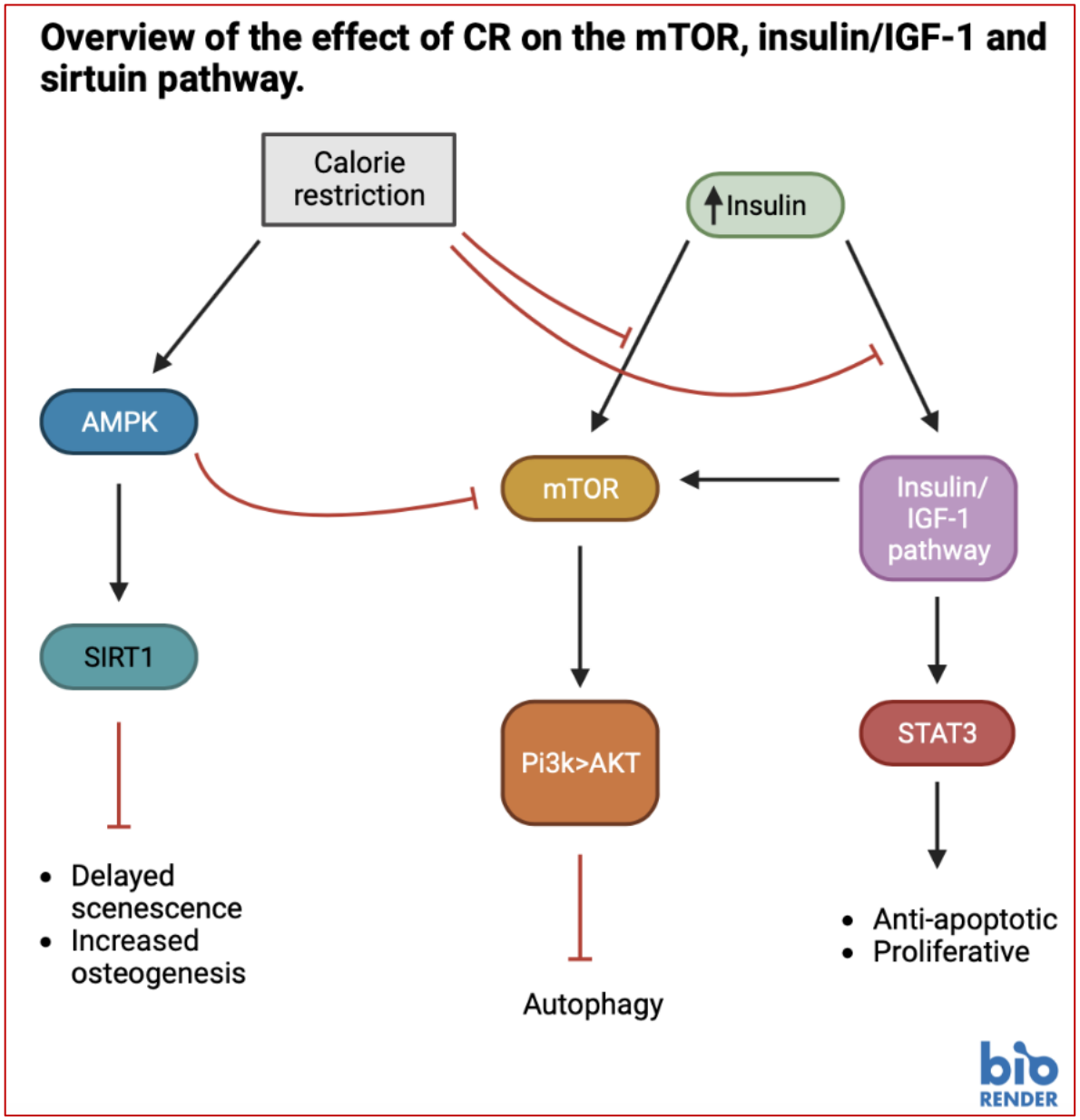

Figure 2. (Adapted from Cantó C et al.J (2011)). Overview of the effect of CR on the mTOR, insulin/IGF-1 and sirtuin pathway. Calorie restriction leads to lower insulin levels and, therefore, inhibits the mTOR pathway and the insulin/IGF-1 pathway resulting in a of anti-apoptotic and proliferative effects and promoting autophagy. CR which could cause metabolic stress upregulates AMPK resulting in the delayed senescence and increased osteogenesis[29].

2.4 Non-human primates and humans

In the late 1980s two studies were conducted in parallel to understand the effect of CR in rhesus monkeys. The university of Wisconsin study reported a significant positive impact of CR on the survival, while on the other hand no significant survival effect was found by the National Institute on Aging study. Mattison et al (2017) described differences in study design that could contribute to the variety in outcome and confirm that health benefits of CR are conserved in monkeys. Also, they suggest that CR mechanisms are likely translatable to human health[28]. In humans, CR has been reported to improve the quality of life and cause weight loss[49, 50]. Furthermore, CR reduced fasting insulin levels, body temperature, resting energy expenditure and oxidative stress, tumors and cardiometabolic risk factors as cholesterol and blood pressure[51,52]. The CALERIE study investigated the short- and long-term effects of CR on cardiometabolic risk factors in healthy, lean or slightly overweight young and middle-aged individuals and found, after 2 years, that CR significantly reduced multiple cardiometabolic risk factors[53]. Therefore, CR might be a promising natural method to increase healthspan and lifespan om humans. However, there is a downside to CR. As a result of continuous appetite, CR, which seems to be a beneficial dietary intervention, is very hard to conserve[9,54].

3. Low protein/high carbohydrate

3.1 Macronutrient balance

The view that CR without malnutrition prolong life has become central knowledge[55–58]. However, recently this view has been challenged by experiments suggesting that specific nutrients rather than energy are responsible for the positive effects on increased lifespan[59,60]. Before, research in the field of nutrition has mainly been focused on the effects of individual macronutrients as for instance sugar, fat, and protein in the search for explanations for obesity, diabetes and other CNCD's[61]. However, there is growing evidence that the balance of nutrients and their interactive effects are more important for healthy aging than the individual, single acting macronutrients[61]. This view resulted in a growing interest in the relationship between an optimal dietary balance and the quality and length of life in humans, although experimental data are lacking and difficult to obtain[62]. A growing number of studies suggest that the restriction of amino acid and protein intake generally decreases aging-related processes and, thereby, increases health and longevity[63]. Moreover, protein restriction has the potential to play an important complementary role in medicine by promoting disease prevention and treatment and by delaying the aging process, at least in part, by stimulating stem cell-based regeneration[21]. The aim of this section is to summarize research on the impact of protein restriction in a low protein, high carbohydrate (LPHC) diet on health and lifespan.

3.2 Optimal protein/carbohydrate balance

Defining what represents a balanced diet is a high priority in nutrition research and the development of the Geometric Framework (GF) now allows to explore how an animal responds to the problem of balancing multiple and changing[10,64]. The GF is a framework which models the optimal balance and amount of nutrients required by an individual animal[65]. The GF considers nutrition as a dimensional space in which the components of the diet, for instance protein and carbohydrate, are represented by the separate axes. Responses of individuals, as for example lifespan, are imposed on this n-dimensional nutritional space by plotting the response surfaces[10,64,66]. *Figure 3* shows an example of a GF showing the relationship between protein, carbohydrate and fat intake versus median lifespan (weeks) in mice.

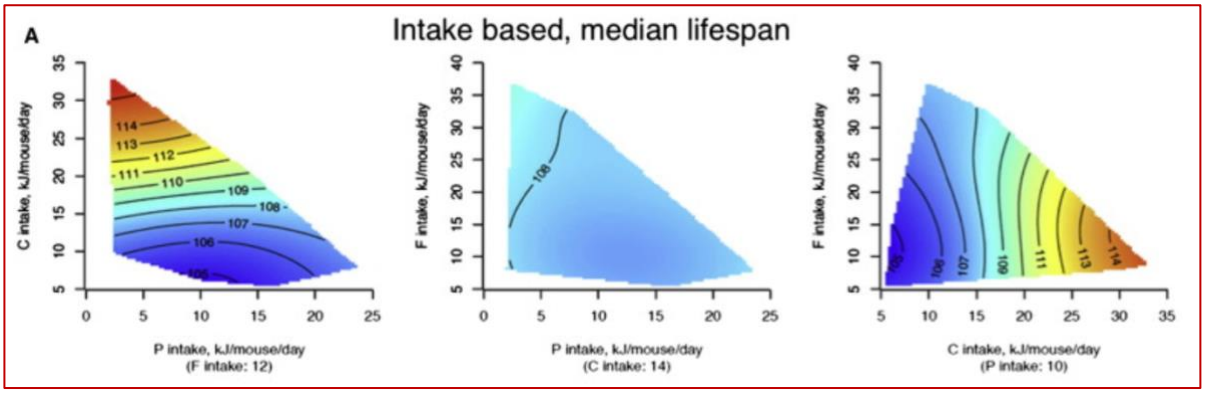

Figure 3. (Taken from Salon-Biet et al. (2014)). Geometric Framework (GF) showing the relationship between protein (P), carbohydrate (C) and fat (F) intake versus median lifespan (weeks) in mice. In all surfaces, red indicates the highest value and blue indicates the lowest value[11].

With this method Salon-Biet et al. (2014) found that median lifespan was greatest for animals whose intakes were low in protein and high in carbohydrate (see *Figure 3A*). Diets which included 5-15% protein and 40-50% carbohydrate resulted in the longest lifespan in mice[61]. Other studies found the same results, reporting that the restriction of protein intake increased healthspan in

rodents[6]. Short-term trials in humans also favor the substitution of protein for carbohydrate as it resulted in decreasing weight, a reduction in blood pressure and an improvement of cardiometabolic biomarkers. Interestingly, multiple studies focused on the impact of the optimal balance of protein and carbohydrate intake on aging also found that the maximalization of lifespan was not influenced by caloric intake[10,12,61].

3.3 Underlying mechanism: mTOR and insulin/IGF-1 pathway

To link the outcomes of their research to the underlying mechanisms of aging, Salon-Biet et al. (2014) measured insulin levels and circulating amino acids. As mentioned before insulin and mTOR are strongly correlated with diet and aging as the mTOR and insulin/IGF-1 pathways progress ageing significantly[8]. Amino acids, especially branched amino acids (BCAA), are key signals for insulin release and mTOR activation[67,68]. BCAA levels correlate positively with daily protein intake levels and insulin values were minimal when protein intake was lowest[61]. Thus, a low protein diet resulted in minimalization of insulin and BCAA levels, which in turn resulted in inhibition of the mTOR and insulin/IGF-1 pathway. Therefore, given the evidence that the activation of the mTOR pathway and the insulin/IGF-1 pathway are pro-aging, the results indicate that a LPHC diet is extending lifespan[6,61,69,70].

3.4 Telomere shortening

As mentioned, the 'hallmarks of aging' are defined as biological processes that underlie aging. In addition to cell senescence, telomere shortening or attrition has been proposed a one of these biomarkers. Telomeres shorten with each mitotic cell division, and this leads eventually to replicative arrest and cellular senescence[71,72]. When investigating the influence of dietary macronutrients on telomere length in aging mice it was found that the longest telomere length occurred in mice on the LPHC diets. As such, the longest telomeres and lifespan were seen in mice maintained on these diets[12]. This supports the notion that the LPHC diet contributes to an extended healthspan.

3.5 Overconsumption

Even though many studies suggest the profound effects of LPHC diets, low protein intake is associated with overconsumption of carbohydrates, which could be defined as total energy intake[13,14]. The 'protein leverage hypothesis' has since been tested in a range of studies, including animals and humans, and it has been shown that restricted protein intake could have a potential role in the contribution of weight gain and obesity[73]. Multiple studies showed that an estimated decrease in protein intake from 14% to 12,5% resulted in an increase of 14% in non-protein energy intake in an effort to maintain constant protein intake[14,74]. In a randomized controlled experimental trial, it was shown that an increased energy intake occurred on diets containing low proportions of energy from protein[17]. This extended energy intake will bring detrimental consequences including weight gain, obesity, diabetes type 2 and fatty liver[15]. It is even suggested that protein leverage might drive the obesity epidemic[14]. In humans the results of a LPHC diet are less well established, however, an LPHC is associated with obesity and fatty liver. Therefore, paradoxically a diet which is higher in protein diet might be more protective for obesity, diabetes and other CNCDs as it will provide a feeling of satiety and, thus, result in reduced food intake[16,17].

Discussion

As identified in this literature review, studies about the dietary intervention's calorie restriction and low protein/high carbohydrate often present divergent conclusions about the benefits or harms of healthy ageing and the maximalization of lifespan. In this review it was attempted to interpret the current knowledge of dietary intervention studies and to draw conclusions, by deciding on which nutritional intervention, CR or LPHC, is most relevant for healthy aging. Although it could be confirmed that nutrition has a powerful impact on ageing and age-related health, this review demonstrates that the issue of whether reduced protein intake or the effect of caloric restriction contributes to lifespan best remains unresolved despite a century of studies.

Here, we characterized the dietary intervention CR and LPHC which impact aging via underlying mechanisms as the mTOR, insulin/IGF-1 and sirtuin pathway[8]. Contrary to the hypothesized association, the found results confirm that CR has a deeply embedded accuracy. Well conducted studies in rodents show that long-term CR has promising effects on the metabolism in relation to aging[27]. CR influences the underlying pathways directly and indirectly which results in a decrease of the hallmarks of aging such as senescence[44]. In addition, the first studies in humans also show positive effects on cardiovascular metabolism[53]. However, these data must be interpreted with caution as the longest time span of any study conducted in humans was two years. It is not relevant to draw conclusions of such a short study about health and lifespan on the long term as short-term randomized controlled trials fail to consider the long-term impact that nutrition really has. On the other hand, the reduction of senescence, which is one of the hallmarks of aging, as a result of CR could be a prediction of extended lifespan[44].

Investigations of the balance of macronutrients, in particular the protein carbohydrate ratio had marked effect on health and lifespan in rodents. Lifespan declined from the maximum as protein intake increased and carbohydrate intake fell[11]. As the protein : carbohydrate ratio increased, there were significant increases in mTOR activation, which is pro-aging[11]. Therefore, a healthy diet is one that keeps mTOR activation and insulin levels low. This is achieved by the LPHC diet which, thus, has life-extending effects. However, the optimal balance between protein and carbohydrate intake is not set and differs between species[10,61]. In addition, it is not clear what the long-term effects are in humans. On the contrary, multiple studies found evidence for increased protein intake. Maximized lifespan, for example, is commonly expected to be a trade off against fertility. A comparison of the lifespan and egg production in relationship to dietary interventions and aging showed that lifetime egg production is maximized at an intermediate protein/carbohydrate intake[10]. These results are in line with those of previous studies and do, therefore, not favor a LPHC diet. Also, a positive relationship between protein intake and bone mineral density was noted which suggests a high protein intake recommendation for older people to manage sarcopenia[11]. Furthermore, a LPHC diet is often associated with overconsumption[13,17]. With the LPHC diet protein targets are not achieved and, therefore, the organism responds with compensatory feeding. Eventually, this results in weight gain and its profound detrimental consequences. Overconsumption could be reduced by an increase in protein intake or a reduction in overall food intake. Opposingly, this reintroduces the question whether the effects of prolonged lifespan are a consequence of a CR or a LPHC dietary intervention[17].

Although the current literature shows promising results of both dietary interventions, further research is needed to establish how nutrient requirements change throughout a lifetime and how different macronutrients interact. In this review, the LPHC dietary intervention was emphasized,

however, the macronutrient fat was not considered. The reason to not take fat into consideration was based on the findings of Salon-Biet et al. (2014), who concluded that high-fat diets had minimal impact on food intake. They found that as the concentrations of either protein of carbohydrate decreased in the diet, chronic food intake increased. In contrast, fat intake continued to increase as the proportion of dietary fat increased. Therefore, the fat content in the diet was mainly unregulated and thus had negligible influence on food intake[11].

In conclusion, to make progression in the improvement of dietary adherence a greater effort should be made to understand the underlying mechanisms of aging in relation to individual variability in responses to a specific dietary intervention. Today, dietary intervention remains the most applicable and cost-efficient means of preventing and treating a wide variety of ageing related diseases for humans. Another point of action is the translation from rodents and other models to humans over the long term. As high protein diets are widely promoted for weight loss and health, it is accurate to take immediate action and to understand the effect of protein intake. Namely, if restricted protein intake eventually leads to a prolonged lifespan, what might be the life shortening consequences of the excessive ingestion of high protein amounts. It is clear that dietary interventions aimed at influencing health or aging require careful evaluation in the context of the underlying mechanisms before solid conclusions can be drawn.

Addendum

Now the whole process of the project is finished, I would like to express my gratitude for my supervisor Cor Calkhoven. I would like to thank you for your effort in guiding me through this project. Your enthusiasm for this subject inspired me and it was fun to explore the complex and varied world of dietary interventions. It was amusing to listen to your stories and explanations, and I tried to incorporate your feedback the best as I could. This project was very valuable to me as it enjoyed taking my own responsibility and, furthermore it allowed me to invent a way to read and summarize comprehensive literature in an efficient manner. Lastly, I liked to dig in this field of Biomedical Sciences to find out whether it is of interest for me and if it might be a subject for me to specialize in in the near future.

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