Bachelors Thesis

BSc Life Science & Technology
Biomedical Sciences
Rijksuniversiteit Groningen ERIBA

The effect of protein restriction on health and disease

A literature review summarizing the effect of protein restriction in humans and criticising the term protein restriction.

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Abstract.

As time progresses, healthcare is getting more and more advanced, leading to increased lifespans and a relative increase in the elderly population. One of the ways to increase lifespan is dietary restriction, which has been studied for decades now. Even with many doubts and uncertainties, it has stood at the centre of ageing research. As mechanisms and optimal nutritional values (specifically for ageing/longevity) remain poorly understood, research is still ongoing.
Protein restriction was recently identified as a potential benefactor towards increasing lifespan, with the promise that it might be able to reduce age-associated diseases. Research on protein restriction claims a 4-fold decrease in cancer prevalence, reduced levels of diabetes and more.
This review summarizes these main findings of protein restriction, explores additional potential benefits of protein restriction, marks the potential disadvantages of protein restriction and criticizes the term ‘’protein restriction’’.

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Introduction.

In the past century, life expectancy has risen drastically due to an increase in medical care and quality (Crimmins, 2015). New ways of increasing the life expectancy of humans are sought after, and especially *healthy* ageing is/was the subject of study in the last decades. One of the ways which showed a lot of promise in ageing healthily was calorie restriction (Al-Regaiey et al., 2016).

Calorie restriction

Calorie restriction is well studied, and the research field has existed for decades already. The first studies which showed promising results were mainly done in model organisms like rodents and non-human primates, with findings suggesting reduced morbidity and increased longevity (Al-Regaiey et al., 2016). Additional studies showed that the genetic background was important in terms of how the CR (calorie restriction) diet exerted its effects (Al-Regaiey et al., 2016). More recently, human studies (analysis of calorie-light culture diets, randomized clinical trials) showed promising results in calorie restriction diets (Most et al., 2017; Al-Regaiey et al., 2016). Diets with reduced calorie intake show many improvements in health, including a reduction in pathogenetic factors for type 2 diabetes, cardiovascular diseases and cancer (Most et al., 2017).

Even though many studies are positive about calorie restriction, there are also critics. Some studies found mixed results, which were mainly attributed to genetics; a diet exerts a different effect on any genetic background (Swindell, 2012). Additionally, there are critics which point out that the rodents (model animals of these studies) were first fed *ad libitum,* which leads to fat rodents who are generally less healthy and develop diseases more quickly and often (Sohal & Forster, 2014). In this way CR is cherry-picked to be healthy, as a reduction in food intake would logically reduce the weight in overweight rats, leading to a reduction in disease (Sohal & Forster, 2014). Even so, it can't be denied that in many cases in both model organisms and humans, calorie restriction has a positive effect on longevity and disease. Molecular mechanisms of calorie restriction are now getting more emphasis, which led to several new insights.

Calorie- and protein restriction and cancer

Recent research has dived into the mechanisms in which CR works, and it is shown to be similar in humans and rodents (Most et al., 2017). In some CR diet studies, especially the methionine-restricted diets, there seems to be a reduction in IGF-1, which in turn protects against cancer in rodents (Dunn et al., 1997; Sonntag et al., 1999; Fontana & Klein, 2007). However, in humans, long-term CR studies did not show a decrease in IGF-1 levels, suggesting the mechanisms in rodents and humans not to be entirely the same (Fontana et al., 2008). One interesting finding is that protein restriction (reducing intake by roughly 40%) for only 3 weeks shows a significant reduction in IGF-1 levels in humans (Levine et al., 2014). This study also shows an increase in cancer with high protein intake under the age of 65, along with a protective role for high protein consumption for people over the age of 65. Cancer is becoming more and more prevalent, partly due to the increase in life expectancy but also due to other lifestyle factors (increase in GDP, obesity, sedentary lifestyle, smoking etc.) (You & Henneberg, 2017). Although cancer treatments are getting a lot of funding, and are improving each year, most cancer treatments put a heavy burden on the body. Since treatments are not 100% effective,

and have many negative side effects, preventing cancer is the best way of ‘’combatting’’ this disease, which is why protein restriction seems to be an interesting dietary approach to do just this.

Protein restriction

Because of the promising results in certain studies, protein restriction now has a name separate from calorie restriction. The name already indicates; protein restriction is the dietary reduction of protein intake, reducing the percentage of calories obtained from proteins in a diet. Protein is known to have several effects on molecular mechanisms in the body, which in turn affect ageing and health. Example mechanisms in which protein restriction could affect the body are mTOR signalling, IGF-1 reduction, and the reduction of several amino acids (amino acid restriction) are discovered to affect cellular stress and tRNA (Mirzaei et al., 2016; Hill & Kaeberlein, 2021).

This review serves as a summary of the health effects of a protein-restricted diet and explores potential additional benefits. It will analyse the role of protein restriction in cancer, diabetes, bone disorder, muscle building, chronic kidney disease and more. It will also give an unbiased opinion on whether protein restriction has the potential as a therapy against certain diseases.

Findings and results.

The history of protein restriction

The idea that *‘’more protein is better’’* has been around for quite some time (and I dare say it still is around today). In 1890, the USDA already set a high standard, recommending 110 grams of protein a day for working men, as it was thought to be the source of muscular energy, combined with research showing that ‘’successful’’ social groups consumed more protein (data which seems somewhat biased, as the primary source of protein was meat, which is expensive, thus more accessible to the rich) (Carpenter, 1986). Later it was found that 55 grams of protein a day were generally sufficient, thus the standards were adjusted (Carpenter, 1986).

Protein deficiencies were often thought to be the cause of diseases, but later research has debunked this since. It is nowadays near impossible to maintain a diet which causes protein deficiency, as the body is known to be able to build its proteins from amino acids, due to the amino acid recycling system (Johansson G, 2018). Bodily proteins are also broken down and sent into the intestinal tract, this is the source of the majority of amino acids absorbed (Johansson G, 2018).

Eventhough there have been massive advances in the medical industry in the past decades, the exact effects of nutritionary composition on disease have not yet been fully unravelled. An example of a dietary component which is not as extensively studied as some others is protein concentration in diets; especially diets containing *low* protein concentrations. Eventhough it has not gained a lot of traction, low protein diets have been used in some diseases. An example of a disease for which low protein diets were prescribed is chronic kidney disease (CKD) (Milovanov et al., 2013). Low protein diets have also been reported to have selective positive effects on people suffering from hepatic encephalopathy (Nguyen & Morgan, 2013).

Lately, the notion that a high percentage of protein in diets is promoting the development of many diseases has gained traction. Eventhough in healthy adults it is not thought to cause any problems, supported by a randomized crossover study done by *Antonio et al.,* long-term longevity and disease studies see a deleterious effect of high protein diets on several diseases (Delimaris, 2013).

Benefits of protein restriction

Many studies have associated excessive protein intake with detrimental effects on the body, or have seen protein restriction to be beneficial for longevity. The following paragraphs will summarize and critique these findings.

Cancer

An article by *Levine et al.* shows a reduction in IGF-1 levels in protein restriction diets. They point to the fact that mice and humans with Growth Hormone Receptor (GHR)/ IGF1 deficiencies show a major reduction in ageing-associated disease and suggest protein restriction to be potentially beneficial in reducing cancer prevalence. Their findings suggest that people reporting a high protein diet have a 4-fold increase in cancer incidence, suggesting that high protein diets increase the chance of cancer significantly (Levine et al., 2014). They do mention that the effects of the protein restriction are abolished or attenuated if the source of protein was plant-based.

Additionally, several articles point to the big role of dietary practices on cancers, with up to 80% of breast bowel and prostate cancers being attributed to them (Delimaris, 2013). Especially a high percentage of protein intake from red meat and processed meats in the diet seem to increase the risk of cancer (Norat & Riboli, 2009). Many other studies also show that meats increase cancer (Yang et al., 2012; Paluszkiewicz et al., 2012; Lippi & Mattiuzzi, 2012).

Interestingly, these are mainly the articles cited by protein restriction articles claiming that protein restriction causes a decrease in cancers, which seems off. The increase in cancers might very well not be because of the proteins in the diets, but because of the many other things found in a red meat diet for example saturated fats, which are also known to cause an increase in several types of cancers (Kafatos et al., 2008). Red meat has other studied mechanisms which are known to increase the chance of developing cancer, such as causing the formation of heterocyclic amines, which require acetylation by p450 enzymes, which have been associated with an elevated risk of large-bowel cancer (Bingham, 1999). Additionally, after consuming red meat, NH3 is formed by bacteria in the bowel, which is a promotor of n-nitroso compounds which are known to cause cancer (Bingham, 1999).

To question the notion of whether it's the dietary protein or other components in red meats, plant and dairy protein should theoretically also increase cancer. Interestingly, an article by *(Naghshi et al., 2020)* showed the opposite to be true, suggesting a negative correlation between plant protein intake and risk of all-cause and cardiovascular mortality, which includes cancer. This article suggests swapping from animal protein to plant protein to increase longevity. The interesting takeaway from this is that it might not necessarily be the protein, but just the source of the protein, since you could very well have a high protein diet without the increased risk of cancer, just by consuming plant-based proteins, and moderating/staying away from red meats as a source for protein intake.

Chronic kidney disease.

CKD is defined as the presence of kidney damage, or as having an estimated glomerular filtration rate of less than 60ml/min per 1.73 square meters for at least 3 months or more (Vaidya & Aeddula, 2021). It gets progressively worse, reducing kidney function to the point where there is a need for dialysis or transplantation of a new kidney (Vaidya & Aeddula, 2021). There are many types of medication in the field already, which include Renin Angiotensin Aldosterone System (RAAS) blockers/statins, which don't cure the disease but delay the progression (Baragetti et al., 2019)
Protein restriction, which in this case is called low protein diets, has been used in CKD for a while, with the aim to reduce the progression of CKD, in combination with RAAS blockers/statins. Although the effectiveness of the low protein diet is discussed as clinical studies show marginal results, it is still in use. A new study by *Baragetti et al., 2019* does show a significant delay in kidney degradation in patients with severe CKD, with low protein diet patients starting dialysis (procedure to remove waste products and excess fluid from the blood when the kidneys stop working properly) on average 24 months later than patients with an unrestricted protein diet.

This is a significant improvement, however, it is not very relevant to the protein restriction increasing longevity for an obvious reason; it is only beneficial in patients suffering from CKD, and thus does not translate to ordinary longevity.

Renal disfunction

High protein intake is considered one of the biggest risk factors for kidney stones, along with low fluid intake (Goldfarb DS; Coe FL, 2015). Protein ingestion causes calcium to be excreted by the kidneys leading to hypercalciuria (Goldfarb, 1988). This happens because protein ingestion increases renal acid excretion, and these acid loads will be buffered by bone, which causes the excretion of calcium in the urine (Goldfarb, 1988). This hypercalciuria can in turn cause the formation of kidney stones (Goldfarb, 1988). Additional mechanisms causing kidney stones include hyperuricosuria, which is in part caused by an animal-protein diet, as meat contains purines which are precursors of uric acid, leading to hyperuricosuria (Kidney Stones, 2013). Many later studies have proven the point that high animal protein ingestion significantly (250%) increases the chance of developing kidney stones (Delimaris, 2013).
Because of this, protein restriction could be beneficial in case of preventing kidney stones, not with the aim to promote longevity, but for some people, it could increase the quality of life. One recurring theme which is interesting to note is that again, the aquisition of proteins from animal sources seems to be the root of the problem, not solely the protein itself. This is proven by the fact that swapping animal proteins for plant-based proteins is a dietary approach intended to reduce the formation of kidney stones.

Bone disorders

Adequate protein intake is positively correlated with bone health as long as adequate calcium is also ingested (Mangano et al., 2013). However, later studies showed too much protein intake causes the bones to be fragile. This is proven and explained through a variety of mechanisms. One of the known mechanisms in which an excess of protein in your diet causes bone fragility is through the calcium balance. Several studies show that an increase in protein consumption leads to an increase in calcium excretion, which was measurable in the urine, and that it led to a negative calcium balance (Kim & Linkswiler, 1979; Licata et al., 1979). A negative calcium balance leads to the activation of PTH (parathyroid hormone) which aims to prevent hypocalcemia (US) et al., 2022). It does this by causing osteoclastic absorption of the bones, resulting in weaker bones, which in turn might lead to osteoporosis. (US) et al., 2022). Additional prospective studies prove this point and found that women who consumed >95 grams of protein a day had an increased risk of forearm fracture relative to women who consumed <68 grams of protein a day (Feskanich et al., 1996).

Interestingly again, a later study investigated whether consuming more vegetable protein relative to animal protein might alleviate these effects; and found that swapping from animal protein to vegetable protein might lower the chance of hip fractures, and decrease bone loss associated with an increase in protein intake (Sellmeyer et al., 2001) The theory they brought forward to explain this difference is that vegetable foods provide base amino acids along with amino acid precursors, while animal food predominantly only provide amino acid precursors (Sellmeyer et al., 2001).

Heart disease and diabetes

Several studies reviewing protein restriction as a therapeutic promise highlighted the decrease in the incidence of ischemic heart disease (IHD) (Mirzaei et al., 2016). Studies about proteins and IHD have indeed shown an increase in the prevalence of IHD in men when on a high protein diet, and theoretically, protein restriction could work in favour to reduce this chance (Preis et al., 2010; Virtanen et al., 2018). As before, there is no correlation between increased incidence of IHD with ingesting more protein coming from vegetable sources. Again, the evidence points to the fact that it is not necessarily the protein which is bad, but the source from which the protein comes.

The same article by *Mirzaei et al.* mentions the potential of protein restriction to reduce diabetes. In the article itself, it gives ‘’a reduction of meat intake’’ as the reason, which doesn't seem like protein restriction, but more like red meat restriction. Other studies, which reportedly see an increase in type 2 diabetes mellitus (T2DM) with an increased protein diet, support the claim that red meat restriction, but not necessarily protein restriction reduces the chance of developing T2DM (van Nielen et al., 2014; Steinbrecher et al., 2010).

Amino acid restriction

Amino acid restriction has not been studied in humans yet, however, in model animals, it has shown a lot of promise.
In rodents, the specific restriction of amino acids is proven to be just as potent in increasing longevity as calorie restriction (Mirzaei et al., 2014). Amino acid restriction improves longevity in part through the modulation of amino acid sensing pathways TOR and GCN2 (Mirzaei et al., 2014). The restriction of 2 amino acids specifically, tryptophan and methionine have shown longevity-promoting effects (Gallinetti et al., 2012). Tryptophan has several effects in rodents, including delaying sexual

maturation and tumour onset, improved hair growth and coat condition, protection against ischemia and injury in kidney and liver, along with an increased health span (Gallinetti et al., 2012; Peng et al., 2012). The benefits of methionine restriction can in part be explained through a decrease in mitochondrial reactive oxygen species, insulin levels, IGF-1 levels and serum glucose, and it contributed to the loss of adiposity by affecting the lipogenic/lipolytic balance (Gallinetti et al., 2012; Minor et al., 2010). The other effect of general amino acid restriction is the activation of the IGF-1 and TOR-S6K, which also likely increases lifespan (Mirzaei et al., 2014).
Other studies show that lifelong restriction of BCAA's (leucine, isoleucine and valine) yields a 30% increase in lifespan and a reduction of frailty in male rats (Richardson et al., 2021). BCAA restriction can additionally improve metabolic health when started in midlife (Richardson et al., 2021).
There are many more effects of amino acid restriction, such as an increase in stress resistance genes, acetyl-COA production which in turn affect epigenetic modifications, and cytoprotection, which can also increase lifespan in model animals (Mirzaei et al., 2016; Mirzaei et al., 2014).
Eventhough not completely relevant to this paper as amino acids are not complete proteins, and since research is not conducted in humans, it still seems an interesting approach to dietary restriction, with good results.

What was not suggested by any articles or reviews, is switching the diet from red meat protein sources to vegetable protein. Nearly all positive effects ‘’protein restriction’’ has yielded could be explained due to the switch from eating an abundance of red meats to eating less of these. This is supported by the fact that swapping from a vegetable protein diet to a protein-restricted diet rarely increased the chance of disease in humans. The articles do mention that the effect of protein restriction is lost when plant-based proteins are used but don't drop the term protein restriction when mentioning the positive effects.

Possible disadvantages of protein restriction.

Protein restriction (predominantly a restriction of consuming proteins from animal sources) seems to have several benefits. Regardless of the positive effects that might be associated with protein restriction, it is equally, if not more important, that restricting protein intake does not harm the body.

Protein restriction in the elderly

As explained earlier, becoming protein deficient is close to impossible due to the body recycling amino acids and proteins in the intestines. Modern-day protein deficiency is rare in first-world countries and has not been a problem in protein restriction trials, as the subjects don't cut out all protein, but restrict the intake (Johansson G, 2018; Delimaris, 2013). Most articles writing about protein restriction do however mention that it is not recommended for the elderly (65+).

Several studies show that the elderly benefit from excessive (more than the RDA >0.8 g/kg body weight) protein intake in several ways, including a reported increase in muscle mass, strength and overall functioning (Wolfe et al., 2008). Other improvements in the elderly of increased protein consumption include potential improvements in other statuses such as immune functioning, wound healing and blood pressure (Wolfe et al., 2008). In the paragraphs above some dangers of excessive protein intake on bone, renal and cardiovascular functions were discussed, and these were also a concern in the elderly. Research shows the opposite, showing that some of these factors might even be

improved upon ingesting more than the RDA. Other studies show that low protein intake is associated with frailty in the elderly (Coelho-Júnior et al., 2018). Many more studies support the increased intake of protein in the elderly, suggesting that protein restriction is not viable in the elderly and would not yield the benefits it reportedly has in adults. When protein *is* restricted in the elderly, even the benefits of protein restriction are lost, such as the reduction in IGF-1 and more (Levine et al., 2014).

Protein restriction and muscle mass

Protein restriction articles mainly focus on longevity, and because of this, they tend to leave out details not related to longevity. An obvious left-out detail would be muscle mass, more specifically gaining muscle/retaining muscle mass, which is described in no protein restriction articles. For people involved in sports or trying to gain muscle mass, this is an important detail. The current defined RDA (recommended daily intake) is 0,8 grams, but this is often misinterpreted as the optimal intake instead of the minimal intake (for which the RDA is designed) (Carbone & Pasiakos, 2019). It is proven that when trying to increase strength and muscle mass, a higher protein diet is beneficial, especially combined with resistance training (Stokes et al., 2018).
A randomized controlled trial shows that in female sporters a high protein diet yields a significant increase in fat-free mass relative to the low protein diet, however, a major difference in strength between low protein diet and high protein diets was not found (Campbell et al., 2018). For aspiring sporters, they do recommend a high protein diet (Campbell et al., 2018). Protein restriction would inhibit the gains of fat-free mass and muscle in sporters and might therefore be deleterious for their personal goals. A no-compromise solution to this would be switching from the protein source, from red meats to a plant-based diet. This would not necessarily decrease the amount of protein consumed and still has a positive effect on longevity.

Conclusion & discussion

Not protein restriction but *animal* protein restriction.

Protein restriction is evident to have many beneficial longevity effects in some cases. Articles write about a reduction in many ageing-associated diseases, including cancer and diabetes, and show a major reduction of both of these diseases in the context of protein restriction.
A 4-fold decrease in cancer can be seen in a protein restriction diet. Many other studies also point to the fact that a high percentage of proteins consumed from red meats increase the risk of cancers. For people eating lots of red meats, protein restriction can thus be beneficial as it reduces the risk of cancer, however, protein restriction is not the only solution. Switching from meat-based proteins to plant-based proteins is a way to keep consuming as many proteins, thus would not be considered protein restriction, but would significantly reduce the chance of cancer. Diabetes shares the same narrative, as protein restriction articles write about a reduction in diabetes when on protein restriction, but show no effect when the proteins are plant-based, suggesting that it is the protein source which is the problem.

Theoretically, protein restriction could also reduce the chance of developing bone disorders, as excessive protein intake (with or without) adequate calcium intake can lead to the weakening of bones due to a calcium imbalance, causing bone disorders such as osteoporosis. Different studies show an increase in hip and forearm fractures in people with a high protein diet compared to a low protein diet. interestingly, even in this case switching to plant-based proteins attenuate these effects, and even reduces the chance of hip fractures.
Protein restriction is proven to be beneficial in people suffering from CKD, with results showing that low-protein diet individuals need dialysis on average 24 months later than patients fed a normal diet. These results are however not interesting to general longevity, as it only concerns people suffering from CKD, and doesn't necessarily affect healthy people.
Other studies show that high-protein diets significantly increase the chance of developing kidney stones, which is not a longevity issue but a quality of life issue. Again, swapping from animal proteins to plant-based proteins is a way to reduce the chance of developing kidney stones.

It feels like the term ‘’protein restriction” is thus a wrong term for the field, and swapping to a term that more accurately resembles the importance of the studies would be preferable, since the benefits are gained not by limiting *protein* intake but to limit the amount of protein ingested from red meat, or other animal sources. The same benefits could be achieved by switching the majority of protein intake towards a plant-based protein diet. Reading abstracts about protein restriction all seems very promising, but after a more thorough analysis, it seems that a reduction of red meat intake is responsible for nearly all of the reported benefits.

Additional reasons for the term protein restriction to be misleading is that it might alert professional sporters, or general people trying to gain muscle. When hearing that protein restriction causes a 4 fold decrease in cancer, they might lower their protein intake, while that is not the best thing to do in their case. For people trying to gain weight in muscle, or lose fat by consuming more protein it can be bad as it might prompt them to eat less protein, while in reality all they need to do is eat less red meat (if longevity is their concern).
Protein restriction in the elderly, as the other articles suggest, is not recommended. It might cause additional muscle wasting and increase frailty. A recommendation would be to *increase* protein intake, preferably plant-based, however, the benefits of this are less pronounced in the elderly.

It would be interesting to see future studies focus more on *protein* restriction, with studies analysing whether only a reduction in plant-based protein could perhaps yield different beneficial effects, instead of also focussing on diet and a reduction of meat intake. Also, strength building while on protein restriction is not well studied, this could also be interesting to study in the meantime.

The restriction of specific amino acids seems interesting to study in humans, as it yields good results in model animals. Human trials have not been conducted, as it is hard to restrict amino acids in normal diets. It is promising, and there is a lot of research still possible in model animals, from carbohydrate/fat/protein ratios in diets to amino acid restriction.

In conclusion, protein restriction seems to have less potential on its own as promised, and a different name would suit the research field better. Specific studies on the effect of only *protein* restriction in humans would give better answers. Amino acid restriction has a lot of potential, but lacks research in humans, and would be interesting to study.

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Acknowledgements

Many thanks to Professor Calkhoven for supervising my thesis writing, providing feedback, and helping me come up with an interesting subject.