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Dietary protein intake and long-term outcomes after kidney transplantation

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Mohammad Yusof Said

COLOPHON

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Introduction and aims

INTRODUCTION AND AIMS

The long-term challenge of kidney transplantation

End-stage kidney disease (ESKD) is a condition in which kidney function is insufficient to sustain metabolic and hemodynamic equilibrium. ESKD thereby causes various and serious medical complications, necessitating renal replacement therapy in order to survive (1). It is a condition that currently affects more than 17,600 people in the Netherlands (2). The most common causes of ESKD are hypertension and diabetes mellitus (3). Renal replacement therapy consists of either dialysis or kidney transplantation. Out of these options, kidney transplantation, if possible, is the preferred treatment: it offers higher quality of life and better survival compared to dialysis, at lower costs (4,5). While capacity for dialysis depends on availability of equipment, the possibility of kidney transplantation depends on availability of an organ for transplantation, which results in waiting lists for those that require kidney transplantation. Currently, in the Netherlands an estimated number of 6000 patients with ESKD are on dialysis and approximately 800 patients wait for a kidney transplantation, for which the average waiting time is between 2 and 3.4 years (2,6). This results in a large burden to patients, their families, and the public health system. Unfortunately, every year a significant number of patients die while waiting for a transplantation. To be specific, in 2019, 67 patients on the waiting list died, of which more than half is younger than 65 years old (6).

One way to reduce this burden is to improve the longevity of current kidney allografts and reduce premature mortality in patients who have already received a kidney transplantation. The main focus in renal transplantation medicine has for a long time been on prevention of the acute complications of kidney transplantation, such as acute rejection and surgical complications. While these improvements have greatly benefited patient survival and reduced morbidity in early patient follow-up, the long-term course shows little progress (Figure 1). Still half of all kidney transplant recipients (KTR) die or experience graft failure within the first decade post-transplantation (7). Prevention of premature mortality and late graft failure is therefore a growing focus of interest.

Mortality and late graft failure in kidney transplant recipients

The leading cause of death in KTR after the first year of transplantation is cardiovascular disease (8–10). KTR often suffer from a cardiovascular disease that has predisposed them to develop kidney injury in the first place, and after transplantation will continue to put them at risk of allograft dysfunction. As mentioned, hypertension and diabetes mellitus are the most common causes of kidney failure in the western world. These comorbidities will often persist after transplantation. Additionally, there are other transplantation-related factors that put KTR at increased cardiovascular risk, such as chronic low-grade inflammation resulting from infections or chronic rejection (12,13).

Figure 1. Kaplan-Meier curves of death-censored kidney allograft survival in Europe, per decade of transplant year. (11)

From: Coemans et al. *Analyses of the short- and long-term graft survival after kidney transplantation in Europe between 1986 and 2015. Kidney International* 2018; 94: 964-973, License number (Elsevier): 4895260846072.

All these risk factors are great contributors to overall mortality and graft failure in KTR. These risk factors have in common that they are not easily reduced with medication or surgery. Also, conditions such as hypertension and diabetes mellitus have a chronic character of which the subsequent complications, such as renal damage, only appear after the conditions have persisted for many years (14,15). Therefore, in recent years focus has shifted towards preventive medicine. Diet is an essential part of preventive and lifestyle medicine and is putatively one of the

oldest modalities to reduce the risk of developing many chronic diseases. For KTR care, however, it may be an insufficiently used therapeutic option.

Protein intake and the kidney

Dietary protein is a source of amino acids and energy. Both are necessary for development and maintenance of most biological processes in the human body. Amino acids are a source of nitrogen, which is estimated to account for approximately 16% of all protein mass (16). Nitrogen balance is the equation of daily nitrogen intake through amino acids in dietary protein minus nitrogen losses through, for example, urinary urea excretion (17). The nitrogen balance is used to calculate the minimum daily protein requirements of an individual. For healthy, adult individuals, the recommended daily allowance of dietary protein is 0.8 g/kg body weight/day, according to the Dutch dietary guidelines (18). For individuals with acute or chronic disease, and for elderly individuals (age >65 years), it is suggested that the recommended daily allowance should be in the range of 1.0-1.2 g/kg body weight/day to prevent wasting (19). Adequate protein intake and metabolism depends not only on the quantity of protein intake, but also the quality of dietary protein. Protein quality regards the digestibility of dietary protein and its amino acid composition. Essential amino acids, e.g. methionine and histidine, rely on sufficient dietary intake. Dietary protein intake from animal sources are generally of higher quality than protein intake from plant sources (20). High quality plant proteins are, for example, soyabean and pea protein which have similar digestibility as animal proteins (21). Certain dietary amino acids are essential substrates for processes that exceed their basic properties as 'building blocks' of protein synthesis, and display biological activity. Arginine, for example, is essential for the formation of nitric oxide (NO), while methionine and cysteine are essential for the formation of hydrogen sulfide (H₂S). These compounds are known as gasotransmitters: small molecules that can have local and distant effects on many biological processes in the human body. Gasotransmitters affect several metabolic as well as hemodynamic processes and are essential factors in the development of cardiovascular disease (22-24).

Obviously, healthy protein intake is needed to maintain biological processes. However, for the patient with kidney disease, protein intake has a double edged sword. Whereas adequate protein intake is required to prevent malnutrition, in patients with CKD protein intake has been associated with adverse effects.

These include the accumulation of protein catabolic products, e.g. urea (25), and increased protein-induced hyperfiltration and proteinuria (26,27). This is of importance, as proteinuria may in turn induce many inflammatory reactions that result in progressive renal fibrosis and function loss (28–30). Previous studies have also found that high protein intake has various other physiological effects on the kidneys, among which a higher renal plasma flow, glomerular filtration rate, and metabolic acid load (31–34). In chronic kidney disease (CKD) stage 3-5 (glomerular filtration rate <25ml/min), patients are advised by the National Kidney Foundation Disease Outcomes Quality Initiative (NFK KDOQI) guideline to reduce their protein intake: a low protein diet providing 0.55-0.60 g/kg body weight/day or lower when supplemented with keto-acids (35). Several large, clinical studies have investigated the effects of a reduction in dietary protein intake (i.e. less than 0.6 g/kg body weight/day) on renal outcomes in patients with CKD. Of these studies, the Modification of Diet in Renal Disease (MDRD) study is most influential (36). Nevertheless, a large Cochrane systematic review (2018) of the literature found that for patients with CKD, a low protein diet (0.55-0.6 g/kg body weight/day) does not significantly reduce the risk for ESKD or death compared to a normal protein diet (0.8-1.0 g/kg body weight/day) and that most included studies had a low level of evidence (37). This systematic review only revealed a benefit from a very low protein diet (0.3-0.4 g/kg body weight/day) compared to a low protein diet (0.55-0.65 g/kg body weight/day) in reduction of risk for the development of ESKD.

However, such a low protein diet is not without controversy, because one of the major risks of a low protein diet is malnutrition. As previously mentioned, proteins provide essential amino acids and energy. It is known that chronic illness promotes a catabolic state which results in reduced muscle mass and other morbidities (38–40). Patients with chronic illness may, therefore, have higher requirements of energy and appropriate amino acids than healthy individuals (40,41). The NFK KDOQI guidelines emphasize the importance of maintaining adequate energy intake for CKD patients on a low protein diet. For KTR, the exact balance between advantages and disadvantages of a low or high protein diet is yet unknown. There are currently no specific dietary guidelines regarding protein intake for KTR other than the general guidelines for the regular, healthy population. Subsequently, it is still unknown whether doctors should advise KTR to reduce or increase their protein intake. Similar to patients with CKD, it is likely that KTR

should be considered to suffer from chronic illness, including ongoing chronic low-grade inflammation, recurrent infections, or the chronic use of medications, in particular immunosuppressants (13,42,43). Therefore, it can be hypothesized that a higher protein intake is required to prevent a catabolic state in KTR. Conversely, the potential risks of a high protein intake as described earlier should not result in decline of renal function and health. Studies on dietary protein intake in KTR are scarce and early studies have provided no clear conclusion to what type of protein is optimal for KTR (44). Motivated by the prodigious investments of patients and caregivers into kidney transplantation and given the important potential of survival and health benefit, there is a serious necessity for proper nutritional guidance regarding protein intake after kidney transplantation.

Scope and outline of the thesis

In this thesis, we aimed to explore several aspects of dietary protein intake in KTR. The first objective was to assess how the quantity of protein intake affects long-term outcomes in KTR. The second objective was to explore how the quality of protein intake with respect to amino acids affects the long-term outcomes in KTR.

To explore the first objective, we studied the association of 24h urea excretion with mortality and graft failure in a large population of clinically stable KTR in **chapter 1**. Urea is a product of the urea cycle and is a byproduct of amino acid metabolism (45). In biochemically stable conditions, urea excretion represents amino acid turnover. Since amino acids are provided through dietary intake, 24h urea excretion in clinically stable subjects is regarded as a reliable estimate of dietary protein intake (46,47). In **chapter 2**, we explored potential mechanisms through which an association of protein intake with mortality and graft failure could exist, e.g. inflammation, malnutrition, and renal function.

The first two chapters explored general protein intake and made no differentiation regarding the source of protein. We were interested in how the quantity of source-specific dietary protein was associated with long-term outcomes in KTR. No consensus exists on whether protein intake from animal sources is better than protein intake from plant sources and vice versa (21,31,32,48). One of the main challenges of studying source-specific dietary protein intake in observational studies, is the accurate estimation of dietary intake. Biomarkers arguably provide better estimations of source-specific dietary protein intake, because they are not

affected by biases that limit Food Frequency Questionnaires (FFQ), such as recall bias (49,50). In **chapter 3**, we aimed to study the association of novel biomarkers of meat intake with FFQ-derived estimations of meat intake. Additionally, we also studied associations of these biomarkers with mortality and graft failure in KTR.

The second objective was to explore the how the quality of protein intake with respect to amino acids affects the long-term outcomes in KTR. We were specifically interested in amino acids present in dietary protein that may give rise to endogenous generation of the previously mentioned gasotransmitters H_2S and NO. These gasotransmitters are important factors in cardiovascular health and disease, and may consequently have an important role in the long-term survival of KTR. In **chapter 4**, we studied the association of urinary sulfate excretion, a biomarker of H_2S bioavailability, with renal function and graft failure in KTR. We also studied its dependence upon dietary protein intake in KTR. Similarly, in **chapter 5**, we explored the associations of NO metabolites with the long-term outcomes mortality and graft failure in KTR. Furthermore, we studied the association of NO with dietary protein intake in KTR.

An interesting aspect of arginine metabolism is generation of asymmetric dimethylarginine (ADMA). ADMA is a relatively new biomarker that has been associated with mortality and morbidity in many populations, especially in those with decreased renal function (51–54). It is produced from the breakdown of proteins with methylated arginine components (55). ADMA is a competitive inhibitor of nitric oxide synthase (56). In Figure 2, an overview is provided of this inhibition. It is hypothesized that this inhibition is responsible for the many pathological effects of ADMA (55,56).

Figure 2. The inhibitory effect of ADMA on Nitric Oxide Synthase.

ADMA: asymmetric dimethylarginine.

We were interested to investigate whether plasma and urinary ADMA would be associated with protein intake in KTR. To date, relatively little is known how ADMA is regulated. Early studies have suggested that ADMA is a ‘uremic toxin’, given that its blood concentration increases with a decreasing renal function (51,55,57). However, balance studies are scarce. In **chapter 6**, we studied the associations of plasma ADMA and 24h urinary ADMA excretion with renal function in a cohort of healthy individuals before and after kidney donation, to assess the isolated influence of change in renal function on ADMA metabolism and renal handling. We also assessed whether KTR have different ADMA homeostasis compared to healthy subjects. Finally, in **chapter 7** we explored the association of ADMA with long term mortality and graft failure and assessed the influence of protein intake on the association.

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The background is a deep teal color with a subtle, grainy texture. In the upper portion, there are several stylized molecular structures composed of yellow dots (atoms) connected by thin yellow lines (bonds). These structures are scattered across the top, with some appearing as small clusters and others as more complex chains. In the lower portion, there is a horizontal, jagged line that resembles a rocky horizon or a cross-section of a geological formation, rendered in lighter shades of teal and white. The overall aesthetic is scientific and artistic.

Part I

Protein intake and long-term outcomes