Risk factors of chronic kidney disease progression: Dutch cohort studies

Esmeijer, K.

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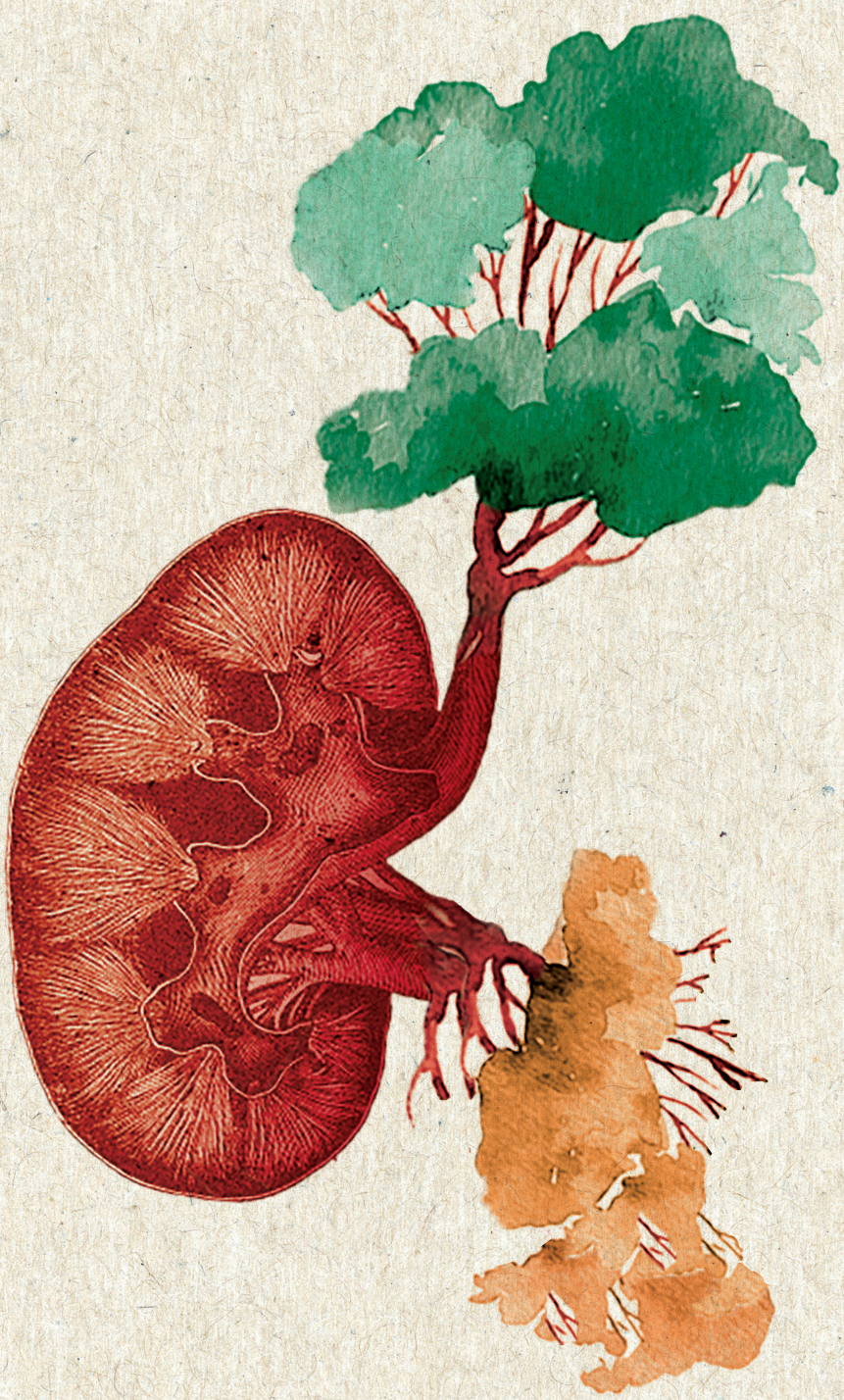

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Risk Factors of Chronic Kidney Disease Progression: Dutch Cohort Studies

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Chapter 1

– Introduction

THE KIDNEY AND KIDNEY DISEASE

Kidneys filter waste products and toxins from the circulation, maintain fluid balance, regulate blood pressure, and are involved in bone mineralisation and erythropoiesis, amongst other things. Disturbances in kidney function may lead to a variety of problems. Healthy kidneys are of vital importance for both our physical and mental wellbeing.

Kidney function is determined by the rate at which the functional units of the kidney, the glomeruli, filter the blood. This glomerular filtration rate (GFR) is measured in mL per minute, usually adjusted for body surface area. Measuring GFR is relatively expensive and time-consuming. Therefore, both in clinical and research setting, GFR is usually estimated (eGFR) by formulae, rather than measured.

Naturally, kidney function deteriorates with age, with about 1 mL/min/1.73m^2 per year after age 40.[1, 2] On average, healthy individuals have a GFR of 100 to 120 mL/min/1.73m^2. Due to the slow rate of kidney function decline, the majority of people will not experience any clinically relevant kidney disease. Kidney function is classified according to chronic kidney disease (CKD) stage 1 to 5 (Table 1). Progression to worse CKD stages is associated with higher risk of end-stage renal disease (ESRD), cardiovascular morbidity, and all-cause and cardiovascular mortality.[3, 4] Clinically relevant CKD is often defined as CKD stage 3 (eGFR <60 mL/min/1.73m^2) or higher. The consequences of CKD stage 3 depend on an individuals' age. For an 80-year old individual CKD stage 3 may be simply the consequence of ageing. In contrast, for a 40-year old individual, further deterioration of kidney function in following decades may eventually lead to ESRD, substantially increasing the risk do die prematurely. Nonetheless, on average a lower kidney function increases cardiovascular and mortality risk also in older populations.[4]

Table 1: Chronic kidney disease (CKD) classification, based on estimated glomerular filtration rate (eGFR).

CKD stage	eGFR (mL/min/1.73m^2)	Terms
1	≥ 90*	Normal or high
2	60 to 89*	Mildly decreased
3a	45 to 59	Mildly to moderately decreased
3b	30 to 44	Moderately to severely decreased
4	15 to 29	Severely decreased
5	< 15	Kidney failure

* CKD stage 1 and 2 are also characterized by albuminuria.

Globally, the prevalence CKD has reached epidemic proportions, and kidney disease is responsible for considerable health care costs and loss of disability adjusted life years. In Europe 11% of the population aged 45 years or older meets the criteria for CKD stage 3.[5] In the United States this is about 44% of the population aged at least 65 years.[6] In 2016, chronic kidney disease caused 1.2 million deaths worldwide, being the 12th cause of death. In contrast, in 1990 chronic kidney disease was the 27th cause of death.[7] The increasing prevalence of CKD can be explained by population ageing, higher prevalence of cardiovascular risk factors, and unhealthier lifestyle the past decades. Given the still continuing trends of ageing, and the difficulty reversing unhealthy lifestyle patterns, the burden of CKD is expected to rise further.

RATIONALE FOR THIS THESIS

Several factors increase the risk of CKD and often risk factors operate complementary. A multitude of risk factors have been investigated in healthy individuals or in relation to cardiovascular disease. But their role in the progression of CKD in patients at high cardiovascular risk is less well documented. The fact that the number of cardiovascular high risk patients will only keep growing, stresses the necessity to explore the role of risk factors in this specific group. An additional challenge in cardiovascular high risk patients is that they often use a variety of cardiovascular drugs. Especially in these medicalized patients, often using multiple cardiovascular medications, it remains unclear whether cardiovascular risk factors still play a significant role in the progression of CKD. This thesis evaluates multiple risk factors for CKD, and focusses mainly, but not solely, on high risk cardiovascular patients on extensive cardiovascular treatment. Ultimately, expanding our knowledge in this field, may facilitate development of treatments specifically tailored to patients at high cardiovascular risk and aid in the development of future guidelines.

OUTLINE OF THIS THESIS

Traditional risk factors

Traditional risk factors for cardiovascular disease, such as diabetes mellitus, hypertension, and cigarette smoking, are important drivers of CKD. Hypertension and diabetes account for about 36% of the age-standardized mortality rate due to CKD. The contribution of diabetes to CKD-related mortality

doubled the past 30 years.[8] Chronic hypertension may lead to progressive glomerular and interstitial fibrosis by increasing glomerular pressure, which results in endothelial dysfunction and loss of adequate auto-regulation.[9] Renal effects of diabetes may present as diabetic nephropathy, a complex and progressive disease characterized by both structural and functional changes of the kidney. These changes encompass basement membrane thickening, glomerulosclerosis, interstitial fibrosis and tubular atrophy.[10] In the early course of diabetic nephropathy, glomerular hyperfiltration is present, which in later stages evolves into a rapid decline of kidney function and progressive proteinuria. Diabetes can be divided into type 1 and type 2 diabetes. Type 2 diabetes, the most prevalent type (about 90% of diabetes cases), usually develops after age 45 as a result of unhealthy lifestyle.[11] In contrast, type 1 diabetes is caused by an auto-immune response against the pancreatic β-cells. Type 1 diabetes generally develops in childhood or adolescence and is much rarer, comprising 5-10% of all diabetes cases worldwide.[11] Though hypertension and diabetes are strong risk factors of CKD, they only explain part of all CKD cases.

Lifestyle

Lifestyle represents another important contributor of CKD progression. Given the increasing burden of cardiovascular morbidity, population ageing, polypharmacy, and rising healthcare costs, non-pharmacological interventions form an appealing opportunity for both prevention and attenuation of chronic diseases such as CKD.

Various components of lifestyle have been gaining increasing interest the past decades, such as obesity, lack of physical activity, smoking, alcohol consumption, and dietary pattern. Obesity is ranked in the top 5 risk factors for death worldwide, and the prevalence of overweight and obesity has been rising steadily.[12] Obesity promotes kidney disease via different mechanisms. It may cause CKD directly by creating an inflammatory environment resulting from accumulation of visceral fat and by inducing chronic hyperfiltration.[13] Obesity also promotes cardiovascular disease, hypertension, and diabetes, thereby indirectly increasing CKD risk.[12] In **Chapter 2** the role of several cardiovascular and lifestyle factors in post-myocardial infarction patients is discussed. We investigated whether type 2 diabetes, hypertension, obesity, and smoking were associated with CKD progression, and whether having more risk factors led to a faster loss of kidney function. In addition, because of the complexity of obesity as a risk factor, it's role in kidney disease progression is investigated in more detail in a separate chapter, **Chapter 3**.

Dietary pattern

As an underrecognized part of lifestyle, dietary pattern is increasingly regarded a potential modifiable risk factor influencing CKD progression. Protein restriction as a reno-protective measure is adapted in nephrology guidelines, mostly for patients with advanced CKD or at high risk for CKD.[14] Dietary protein may damage the kidney by mechanical stress, and by promoting glomerular hyperfiltration through dilation of the afferent glomerular arteriole, thus increasing glomerular pressure.[15] Protein can be of animal or plant sources, and some studies suggested that protein from animal sources is more detrimental than protein from plant sources.[16] However, firm evidence regarding differential effects of animal and plant protein is lacking. Moreover, no nephrological recommendations exist regarding protein intake in individuals with a normal or slightly lower kidney function. In **Chapter 4** the potential role of high protein intake on the rate of kidney function decline is investigated in post-myocardial infarction patients.

Type 1 diabetes

Type 1 diabetes, starting at young age, comes with a 7% cumulative risk of developing ESRD within 30 years. Though dialysis postpones death from ESRD, kidney transplantation substantially improves life expectancy and quality of life.[17, 18] Additionally, simultaneously transplanting a pancreas and a kidney would not only partially restore kidney function, but also restores endogenous insulin production. The latter abolishes the need for insulin medication and prevents further progression of diabetic complications. However, transplanting a pancreas in addition to a kidney also increases risk of complications and peri-operative mortality.[19] To date, it remains controversial whether a simultaneous pancreas kidney transplantation should be preferred over transplanting a kidney alone. In **Chapter 5**, we used nationwide registry data of all type 1 diabetes patients from The Netherlands requiring renal replacement therapy, to compare survival after simultaneous pancreas kidney transplantation, kidney transplantation from a living donor, and kidney transplantation from a deceased donor.

Dyslipidemia

Hypercholesterolemia is also regarded a traditional cardiovascular risk factor, but in contrast to diabetes and hypertension, seems to be of minor importance for CKD progression.[20] Interestingly, cholesterol-lowering drugs (statins) have been shown to beneficially affect kidney function, independent of cholesterol levels. Owing to these pleiotropic effects, statins are nowadays routinely prescribed to CKD patients, and these drugs are in the top 5 of most prescribed

medications worldwide.[21, 22] Till date it remains unknown which statin and dosage, if any, has superior reno-protective properties. **Chapter 6** provides a network meta-analysis specifically addressing this question.

Acute kidney injury

CKD may also be preceded by acute kidney injury (AKI). AKI is a sudden episode of kidney failure, accompanied by rising serum creatinine levels and oliguria, and increases risk of ESRD and mortality. AKI often results from medical care, such as nephrotoxic medication or peri-operative hypoperfusion. The latter is especially relevant for cardiovascular patients. Cardiovascular disease may lead to coronary artery stenosis and myocardial infarction. Post-myocardial infarction patients have a two-fold faster annual kidney function decline, compared to the general population.[23] More importantly, cardiac surgery poses an additional risk. During cardiac bypass surgery a patient's circulation is maintained by a heart-lung machine (cardiopulmonary bypass), increasing AKI risk due to renal hypoperfusion. Depending on the cause, AKI may be difficult to treat, especially when it is not diagnosed at an early stage. At this time, there are no routinely used biomarkers available for the early identification of AKI, but recently several potentially useful markers have been described. **Chapter 7** focusses on these new biomarkers, IGFBP7 and TIMP-2, and considers their potential for predicting AKI after cardiac surgery. Early identification of AKI facilitates more effective treatment and reduces the risk of progression to CKD.

Birth weight

Finally, chronic diseases such as CKD may originate from early fetal life. The Barker hypothesis states that fetal undernutrition during gestation impacts a multitude of developmental processes, leading to higher susceptibility to both physical and mental health problems in later life.[24] For instance, individuals with low compared to normal birth weight have been shown to be more susceptible to develop diabetes, hypertension, and obesity. In relation to CKD, Brenner hypothesized that individuals with a low compared to normal weight at birth, have relatively less nephrons, which over time leads to chronic hyperfiltration.[25] Additionally, relatively smaller kidneys have less overcapacity to compensate in case of kidney damage. However, evidence of a causal association between birth weight and CKD is lacking, since current observational studies on this subject are limited by confounding. In **Chapter 8** we discuss the influence of low birth weight on kidney function at middle-age in a cohort of healthy individuals. To better estimate a causal relation between birth weight and kidney function, we performed two-sample Mendelian randomization analyses using 59 genetic variants associated with birth weight as instrument. Mendelian randomization

exploits the fact that an individuals' genetics are randomly distributed during conception, thus mimicking the randomization procedure of a randomized trial.

SUMMARY OF AIMS PER CHAPTER

Chapter 2 To investigate the association of classic cardiovascular risk factors, and the number of risk factors, with annual kidney function decline, in post-myocardial infarction patients.

Chapter 3 To investigate the association of obesity with annual kidney function decline, in post-myocardial infarction patients.

Chapter 4 To investigate the role of dietary intake of total protein, and protein from animal or plant sources, regarding annual kidney function decline, in post-myocardial infarction patients.

Chapter 5 To investigate in type 1 diabetes patients whether simultaneous pancreas kidney transplantation leads to better survival compared to a kidney transplantation alone, either from a deceased or living donor.

Chapter 6 To investigate the effect of statin use on the rate of kidney function decline and progression of proteinuria, and to gain insight into which statin would be a superior choice, from a kidney perspective.

Chapter 7 To investigate the potential added value of two novel urinary biomarkers, IGFBP7 and TIMP-2, in predicting acute kidney injury, in post-cardiac surgery patients.

Chapter 8 To investigate the association of low birth weight with kidney function at middle-age, using three different methodological approaches. In the Netherlands Epidemiology of Obesity study we performed regression analyses, and two-sample Mendelian randomization analyses using a genetic risk score for birth weight as instrument. In publicly available data we performed a two-sample Mendelian randomization study using summary level data.

Chapter 9 A general discussion about the main conclusions of the various chapters in this thesis, and the implications for clinical practice and future directions.

OVERVIEW OF USED DATA SOURCES

Alpha Omega Cohort (AOC)
The AOC is a prospective cohort of 4837 Dutch patients, aged 60-80 years, with a clinically diagnosed myocardial infarction up to 10 years before study entry. Patients were on standard cardiovascular drug treatment, in accordance with the latest international guidelines. During the first 41 months of follow-up, patients took part in an experimental study of low-dose omega-3 fatty acids (Alpha Omega Trial).[26] The trial started in 2002 and ended in 2009. Major exclusion criteria were severe heart failure, unintended weight loss of at least 5 kg the previous year, and diagnosis of cancer with a life expectancy less than 1 year. For the analyses in **Chapters 2, 3, and 4**, we included all patients with available serum samples at baseline and after 41 months of follow up, about half of the cohort. Since this selection was a random sample of the total study cohort, no bias was introduced.

Netherlands Organ Transplantation Registry (NOTR) and Renal Replacement Registry in The Netherlands (RENINE)
The NOTR is a registry of kidney transplant patients from all eight Dutch kidney transplant centers. The NOTR is a mandatory registry, coordinated by the Dutch Transplant Foundation. The registry contains information on various donor and recipient characteristics, and is updated annually. The RENINE database contains data of all patients with renal failure who need renal replacement therapy. Registration of patients is mandatory for dialysis centers, in order to receive funding. Data quality of the NOTR and RENINE is checked periodically by on-site polls, application rules, and cross checks between both registries. In **Chapter 5**, both registries were linked, resulting in a combined dataset including all Dutch type 1 diabetes patients who commenced renal replacement therapy (dialysis or transplantation) between January 1986 and January 2016 (n=2833).

Cohort of elective cardiac surgery patients at the Intensive Care Unit
This single-center cohort included 812 consecutive patients aged 18 years or older, undergoing elective cardiac surgery at the Leiden University Medical Center, The Netherlands, between December 2006 and August 2010. Exclusion

criteria were pregnancy, active infection, and emergency surgery. According to usual clinical practice, after cardiac surgery patients stayed in the intensive care unit (ICU) for post-operative care. When hemodynamic and respiratory stable, patients were transferred to the thoracic surgery ward. In **Chapter 7**, we analyzed all patients with a complicated ICU stay, defined as staying at the ICU at least 48 hours, and chronologically matched an equal number of patients with an uncomplicated ICU stay.

Netherlands Epidemiology of Obesity study (NEO)
Data of the NEO study, conducted between 2008 and 2012, was used in **Chapter 8**. It is a population-based, prospective cohort study designed to investigate pathways that lead to common disorders.[27] The NEO study included 6671 individuals aged 45–65 years, with an oversampling of overweight or obese individuals. Men and women aged 45-65 years with a self-reported body mass index (BMI) ≥27 kg/m^2 living in the greater area of Leiden were eligible to participate. In addition, all inhabitants aged 45-65 years from one municipality (Leiderdorp) were invited to participate irrespective of their BMI, allowing a reference distribution of BMI. To maintain generalizability towards the general population, analyses in the NEO study were weighted towards the BMI distribution of the general Dutch population.

Summary statistics data: GWAS on birth weight, CKDgen consortium
The instrument for birth weight that we used for Mendelian randomization analyses in **Chapter 8** was based on 59 genetic variants reaching genome wide significance in a Genome-Wide Association Study (GWAS) on birth weight published by Horikoshi *et al.* in 2016.[28] The GWAS included birth weight data from 37 studies comprising 153,781 individuals of multiple ancestries.

In the two-sample Mendelian randomization analyses using summary level data in **Chapter 8**, we used publicly available data from the CKDgen consortium on the associations of each genetic variant with eGFR.[29] The CKDgen consortium includes 133,814 participants of European ancestry from 70 population-based studies, with mean age between 50-60 years and a 5-20% CKD prevalence, defined as an eGFR <60 mL/min/1.73m^2.

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Chapter 2 –

Cardiovascular risk factors accelerate kidney function decline in post-myocardial infarction patients: The Alpha Omega Cohort study

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ABSTRACT

Background: Impaired kidney function is a robust risk factor for cardiovascular mortality. The age-related annual kidney function decline after age 40y of 1.0 mL/min/1.73m^2, is doubled in post-myocardial infarction (MI) patients. We investigated the impact of the number of cardiovascular risk factors (including unhealthy lifestyle) on annual kidney function decline, in 2426 post-MI patients (60-80y) of the prospective Alpha Omega Cohort study.

Methods: Glomerular filtration rate was estimated by serum cystatin C ($eGFR_{cysC}$) and combined creatinine-cystatin C ($eGFR_{cr-cysC}$), using the CKD-EPI equations. Data were analysed by multivariable linear and logistic regression.

Results: At baseline, mean (SD) $eGFR_{cysC}$ and $eGFR_{cr-cysC}$ were 81.5 (19.6) and 78.5 (18.7) mL/min/1.73m^2, respectively. Of all patients, 79% were men, 19% had diabetes, 56% had high blood pressure (≥140/90 mmHg), 16% were current smokers, 56% had high serum low-density lipoprotein (LDL ≥2.5 mmol/L), and 23% were obese (body-mass index ≥30.0 kg/m^2). After multivariable adjustment, the additional annual $eGFR_{cysC}$ decline (95%-CI) was in patients with *vs* without diabetes -0.90 (-1.23; -0.57) mL/min/1.73m^2, in patients with high *vs* normal blood pressure -0.50 (-0.76; -0.24), in obese *vs* non-obese patients -0.31 (-0.61; 0.01), and in current compared to non-smokers -0.19 (-0.54; 0.16) mL/min/1.73m^2. High LDL was not associated with accelerated $eGFR_{cysC}$ decline. Similar results were obtained with $eGFR_{cr-cysC}$.

Conclusions: In older stable post-MI patients without cardiovascular risk factors, the annual kidney function decline was -0.90 (-1.16; -0.65) mL/min/1.73m^2. In contrast, in post-MI patients with ≥3 cardiovascular risk factors, the annual kidney function decline was 2.5-fold faster: -2.37 (-2.85; -1.89) mL/min/1.73m^2.

INTRODUCTION

The incidence of chronic kidney disease (CKD) shows an increasing trend worldwide.[1] Impaired kidney function is a robust and independent risk factor for cardiovascular and all-cause morbidity and mortality.[2] In industrialized countries, in healthy individuals after age 40y, kidney function gradually declines annually about 0.8 to 1.0 mL/min/1.73m^2.[3, 4] In contrast, post-myocardial infarction (MI) patients have an accelerated kidney function decline of about 2.2 mL/min/1.73m^2 per year, and are thus more prone to develop CKD.[5]

Classic modifiable cardiovascular risk factors such as hypertension and diabetes are important drivers for the development of CKD.[6-10] The association between elevated low-density lipoprotein (LDL) levels and kidney function decline is less clear.[11] Lifestyle factors, such as smoking of cigarettes and adiposity, may increase the risk of hypertension and diabetes. All previous mentioned cardiovascular risk factors can have an unfavourable effect on kidney function owing to increased inflammation, oxidative stress, endothelial dysfunction, and disturbed coagulation. For example, accumulation of visceral adipose tissue may lead to increased production of inflammatory mediators by adipocytes, which may contribute to glomerular and interstitial fibrosis.[12, 13]

Survival after MI has been improving, as a result of improved health care and pharmaceutical treatment. These trends, together with the global tendency towards a less healthy lifestyle and population aging, have resulted in a considerable pool of patients at high risk for CKD.[14] Little is known about the beneficial effect of optimal treatment of cardiovascular risk factors and healthy life style on kidney function decline in post-MI patients. Since adequate drug-treatment of cardiovascular risk factors and modest lifestyle alterations are achievable and may retard kidney function decline in post-MI patients, we studied the association of modifiable cardiovascular risk factors (including lifestyle) in older stable post-MI patients of the Alpha Omega Cohort.

METHODS

Participants

This is a secondary analysis of the prospective Alpha Omega Cohort study (ClinicalTrials.gov no. NCT03192410). We included patients from the Alpha Omega Trial, a randomized controlled multi-center trial of omega-3 (n-3) fatty acids supplementation in 4837 patients with a verified history of MI. Patients were aged 60-80 years, and were receiving state-of-the-art antihypertensive, antithrombotic and lipid-modifying drug treatment, according to the international guidelines, as described elsewhere.[15, 16] The trial started in 2002 and ended in 2009. Patients with severe heart failure (NYHA stage IV) were excluded. For the present observational study, patients were selected from whom non-fasting blood was drawn at baseline and after 41 months. Owing to financial constraints two blood samples were available only for 2426 patients (50% of the cohort, i.e. those randomized before August 2005). Of all patients randomized prior to August 2005 (n=2918), 233 patients deceased during follow-up, and 259 patients had missing blood samples or refused participation (Supplementary Figure S1). This study was conducted in accordance with the Helsinki Declaration and was approved by a central medical ethics committee in the Netherlands. Written informed consent was obtained from all patients. Design and reporting of the current study was performed in accordance with the STROBE Statement for cohort studies.[17]

Data collection

Patients were interviewed and physically examined by trained research nurses at baseline and after 41 months. Standardized blood handling procedures for the Alpha Omega Trial are described in detail elsewhere.[18, 19] Lipid, glucose and high-sensitivity C-reactive protein (hsCRP) levels were determined as described elsewhere.[20] Information on demographic variables, lifestyle habits, current health status, and medical history were collected by self-administered questionnaires as previously described in detail.[18] Questionnaires were checked by research nurses. Information on smoking of cigarettes was obtained by self-reported questionnaires, and was dichotomized into current smoking *vs* non-smoking (former or never smoking). Alcohol consumption was dichotomized into at least 1 glass/week *vs* less than 1 glass/week. Systolic and diastolic blood pressure (1st and 5th Korotkoff sound, respectively) were measured at the left upper arm with the patient seated, after a 10 min. seated rest, using an automatic device (Omron HEM-711, Omron Healthcare Europe B.V., Hoofddorp, The Netherlands). The average of two blood pressure measurements was taken. High blood pressure was defined as inadequately controlled blood pressure

according to the latest recommendations of the international guideline of the European Society of Cardiology: systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg.[21] Diabetes mellitus was considered present in case of a self-reported physician diagnosis, use of glucose-lowering drugs, and/or hyperglycemia. Hyperglycemia was defined as serum glucose ≥7.0 mmol/L for patients who had fasted 4 hours or ≥11.1 mmol/L for non-fasting patients. Serum LDL was calculated using the Friedewald formula.[22] High LDL was defined as serum level ≥2.5 mmol/L.[23] Body-mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Obesity was defined as a BMI of ≥30.0 kg/m^2, according to World Health Organization guidelines.[24] Medication was coded according to the Anatomical Therapeutic Chemical (ATC) Classification System.

Kidney function assessment

At baseline and 41 months follow-up, serum cystatin C (cysC) was measured from stored blood samples in a central laboratory. We used calibrators and assays of the same lot code, which was stable (no downward drift).[25] Serum creatinine (cr) was measured by the modified kinetic Jaffé method, as previously described in detail.[25] We estimated glomerular filtration rate based on cystatin C ($eGFR_{cysC}$) and combined creatinine-cystatin C ($eGFR_{cr-cysC}$) at baseline and after 41 months, using the CKD-EPI equations from 2012, taking into account age, sex and race.[26] Both $eGFR_{cysC}$ and $eGFR_{cr-cysC}$ are regarded superior measures of kidney function compared to eGFR based on creatinine alone. In the main analyses we use $eGFR_{cysC}$ as outcome, results for $eGFR_{cr-cysC}$ as outcome are reported in the supplements. From each individual, eGFR decline was calculated by subtracting the eGFR at baseline from the eGFR after 41 months. Assuming a linear decline over time, we then estimated the annual kidney function decline. Rapid kidney function decline was defined as an annual decline of >3 mL/min/1.73m^2.[27, 28]

Data analysis

Baseline characteristics are presented for all patients and according to the number of cardiovascular risk factors. Baseline data are presented as mean (SD), median (interquartile range), or number (percentage) when appropriate. The following data were missing: LDL cholesterol (n=116), BMI (n=4), level of education (n=4), blood pressure (n=3), alcohol consumption (n=3). We accounted for missing data by multiple imputation, using five imputations, and including all relevant baseline variables and the outcome in the model.

We used analysis of covariance (ANCOVA) to compare annual $eGFR_{cysC}$ decline rates for presence *vs* absence of a priori selected cardiovascular risk factors (including unhealthy lifestyle): diabetes, high blood pressure, high LDL levels,

current cigarette smoking, and obesity. In addition, we used multivariable logistic regression to estimate for each cardiovascular risk factor the risk of rapid kidney function decline. In all analyses, we used patients without the cardiovascular risk factor as the reference. All analyses are presented crude and adjusted for potential confounders: age, sex and three dummy variables for the four n-3-fatty acid treatment groups of the Alpha Omega Trial (model 1). In model 2 we adjusted in addition to model 1, for alcohol use (less than *vs* at least one glass/week), level of education (three dummy variables), and the five a priori selected cardiovascular risk factors.. Analyses for obesity were not adjusted for diabetes, high blood pressure and high LDL, because these factors are in the causal pathway between obesity and kidney function decline. Analyses were not adjusted for baseline eGFR, because baseline-adjustment in models with change-scores as dependent variable results in biased and inflated estimates.[29] We explored the presence of effect modification between age or sex and the modifiable risk factors with regard to kidney function decline by including interaction terms in our linear regression models. Furthermore, we repeated analyses in strata of baseline eGFR (eGFR <60, 60<90, ≥90 mL/min/1.73m^2).

Finally, we calculated the rate of kidney function decline and risk of rapid kidney function decline according to the number of cardiovascular risk factors present in each patient. In these analyses we included diabetes, high blood pressure, current smoking, and obesity. High LDL was excluded because of lack of evidence that modifying LDL level affects cardiovascular risk.[30] Patients without cardiovascular risk factors have by definition an optimal cardiovascular risk profile and a healthy lifestyle, e.g. are considered being optimally treated for the included risk factors according to the latest guidelines of the European Society of Cardiology: blood pressure <140/90 mmHg, no diabetes, never smoked cigarettes or ceased smoking, and no obesity (BMI<30 kg/m^2).[31] A linear trend was evaluated by including a variable representing number of cardiovascular risk factors into the linear regression model.

Sensitivity analyses

We repeated all analyses without multiple imputation, using a complete case analysis. Next, we repeated the analyses adjusting for continuous instead of dichotomized variables, e.g. for BMI instead of obesity, and for systolic blood pressure instead of high blood pressure. Main analyses were repeated after adjustment for time since MI, hsCRP levels or use of RAS blocking drugs. We repeated the analyses in patients persistently (at baseline and after 41 months of follow-up) using RAS blocking drugs. Finally, we repeated the main analyses using $eGFR_{cr-cysC}$ as outcome. In these analyses we excluded 82 patients of whom serum creatinine was not available due to technical failure or analytical

disturbance.[25] We considered two-sided P-values <0.05 statistically significant. All analyses were performed using SPSS 23.0 (SPSS, Inc., Chicago, IL).

RESULTS

Baseline characteristics

Baseline characteristics of all patients (n=2426), and stratified for the number of cardiovascular and lifestyle risk factors, are presented in Table 1. The mean (SD) age of the total study cohort was 68.9 (5.4) years, 79.4% were men, median time since MI was 4.0 years, mean (SD) $eGFR_{cysC}$ and $eGFR_{cr-cysC}$ were 81.5 (19.6) and 78.5 (18.7) mL/min/1.73m^2, respectively. Of all patients, 23% were obese, 16% were current smokers, 67% were former smokers, 44% had a blood pressure within the target range, 87% used anti-hypertensive medication, 54% used RAS blocking drugs of whom 92% persisted on RAS blocking drugs, and 19% had diabetes of whom 71% used glucose lowering medication. Finally, 44% of patients had normal LDL, 85% used statins, of whom 95% persisted on statins. Of all patients with high LDL (n=990) at baseline, 10% started with a statin during follow-up.

Table 1: Baseline characteristics of 2426 post-MI patients of the Alpha Omega Cohort according to the number of cardiovascular risk factors (obesity, high blood pressure, diabetes, current smoking).

		Number of cardiovascular risk factors			
	All patients n=2426	0 n=598	1 n=1088	2 n=573	≥3 n=167
Age, y	68.9 ± 5.4	68.3 ± 5.3	69.6 ± 5.4	68.5 ±5.5	68.9 ± 5.4
Men, no. (%)	1927 (79.4)	497 (83.1)	878 (80.7)	430 (75.0)	122 (73.1)
Race, white, no. (%)	2398 (98.8)	589 (98.5)	1078 (99.1)	567 (99.0)	164 (98.2)
Time since MI, y	4.0 (2.0–6.4)	3.3 (1.6–5.9)	4.0 (1.9–6.5)	4.4 (2.4–6.6)	4.8 (3.1–7.4)
Educational level, no. (%)					
Only elementary/low	1374 (57.0)	319 (53.6)	603 (55.7)	353 (62.0)	99 (59.6)
Moderate	738 (30.6)	190 (31.9)	344 (31.8)	152 (26.7)	52 (31.3)
High[a]	300 (12.4)	86 (14.5)	135 (12.5)	64 (11.2)	15 (9.0)
Current smoking, no. (%)	386 (15.9)	0	100 (9.2)	196 (34.2)	90 (53.9)
Alcohol consumption,[b] no. (%)	1759 (72.5)	450 (75.3)	813 (74.7)	390 (68.1)	106 (63.5)
Obese,[c] no. (%)	554 (22.8)	0	146 (13.4)	268 (46.8)	140 (83.8)
Body-mass index, kg/m^2	27.7 ± 3.6	26.0 ± 2.3	27.1 ± 3.1	29.3 ± 4.1	32.1 ± 3.5
High blood pressure,[d] no. (%)	1064 (43.9)	0	744 (68.4)	457 (79.8)	161 (96.4)
Systolic blood pressure, mmHg	143.3 ± 21.4	125.1 ± 10.3	147.6 ± 21.6	150.8 ± 19.6	154.8 ± 17.7
Diastolic blood pressure, mmHg	81.4 ± 10.8	75.1 ± 7.8	83.1 ± 10.9	83.2 ± 10.8	86.3 ± 9.4
Anti-hypertensive drugs,[e] no. (%)	2111 (87.0)	502 (83.9)	954 (87.7)	507 (88.5)	148 (88.6)
ACE inhibitors/ATII blockers	1309 (54.0)	311 (52.0)	576 (52.9)	327 (57.1)	95 (56.9)
Beta blockers	1585 (65.3)	371 (62.0)	718 (66.0)	385 (67.2)	111 (66.5)
Calcium channel blockers	467 (19.2)	111 (18.6)	200 (18.4)	117 (20.4)	39 (23.4)
Diuretics	500 (20.6)	99 (16.6)	198 (18.2)	162 (28.3)	41 (24.6)
Diabetes,[f] no. (%)	449 (18.5)	0	98 (9.0)	225 (39.3)	126 (75.4)
Plasma glucose,[g] mmol/L	6.0 ± 2.0	5.4 ± 1.0	5.7 ± 1.4	6.7 ± 2.4	8.2 ± 3.2

Table 1: Continued

Glucose lowering drugs,[h] no. (%)	320 (13.2)	0	72 (6.6)	169 (29.5)	79 (47.3
Oral glucose lowering drugs	253 (10.4)	0	56 (5.1)	135 (23.6)	62 (37.1)
Insulin analogues	107 (4.4)	0	25 (2.3)	52 (9.1)	30 (18.0)
Serum LDL,[i] mmol/L	2.74 ± 0.80	2.68 ± 0.81	2.74 ± 0.79	2.75 ± 0.79	2.86 ± 0.87
Lipid modifying drugs,[j] no. (%)	2089 (86.1)	518 (86.6)	938 (86.2)	485 (84.6)	148 (88.6)
Statins	2073 (85.4)	516 (86.3)	933 (85.8)	478 (83.4)	146 (87.4)
Anti-thrombotic agents,[k] no. (%)	2368 (97.6)	583 (97.5)	1060 (97.4)	560 (97.7)	165 (98.8)
Serum hsCRP, mg/L	1.7 (0.8–3.6)	1.2 (0.6–2.8)	1.5 (0.8–3.3)	2.3 (1.1–4.6)	2.9 (1.2–5.2)
Serum cystatin C, mg/L	0.97 ± 0.24	0.93 ± 0.20	0.97 ± 0.24	1.01 ± 0.28	0.97 ± 0.24
Serum creatinine,[l] µmol/L	90.1± 29.3	89.2 ± 25.0	90.0 ± 30.4	92.7 ± 32.1	82.1 ± 26.1
$eGFR_{cysC}$,[m] mL/min/1.73m^2	81.5 ± 19.5	84.9 ± 17.9	81.0 ± 19.3	78.7 ± 20.8	81.7 ± 20.5
$eGFR_{cr\text{-}cysC}$,[m] mL/min/1.73m^2	78.5 ± 18.7	80.8 ± 17.1	78.4 ± 18.6	75.8 ± 19.8	80.1 ± 19.7

ACE, angiotensin-converting enzyme; ATII, angiotensin II; cr, creatinine; cysC, cystatin C; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein; MI, myocardial infarction.
Data are reported as number of patients (%), mean ± SD or median (interquartile range).
[a] Higher vocational education or university.
[b] At least 1 glass/week.
[c] Body mass index ≥30 kg/m^2.
[d] Systolic blood pressure of ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg, irrespective of use of blood pressure lowering drugs.
[e] Blood pressure-lowering drugs: Anatomical Therapeutic Chemical Classification System (ATC) codes C02, C03, C07, C08, and C09.
[f] Self-reported diagnosis by a physician, use of glucose-lowering drugs, or hyperglycemia.
[g] To convert the values for glucose to mg/dL, divide by 0.05551.
[h] Glucose lowering drugs: ATC code A10, A10A, A10B, A10X.
[i] To convert the values for LDL-cholesterol to mg/dL, divide by 0.02586.
[j] Lipid-modifying drugs: ATC code C10, C10AA.
[k] Anti-thrombotic agents: ATC code B01.
[l] To convert the values for creatinine to mg/dL, divide by 88.40.
[m] $eGFR_{cysC}$ and $eGFR_{cr\text{-}cysC}$ based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations from 2012.

Kidney function decline

After 41 months of follow-up mean [95%-confidence interval (CI)] $eGFR_{cysC}$ decline for all patients was -4.62 (-5.06; -4.18) mL/min/1.73m^2, corresponding to an annual decline of -1.34 (-1.47; -1.21) mL/min/1.73m^2. Men and women had annual kidney function decline rates of -1.45 (-1.59; -1.31) and -0.91 (-1.19; -0.63) mL/min/1.73m^2, respectively. Patients younger than 70 years *vs* 70 years or older had annual kidney function decline rates of -1.15 (-1.32; -0.98) and -1.60 (-1.80; -1.41) mL/min/1.73m^2, respectively. After multivariable adjustment (model 2), patients without or with diabetes had an annual $eGFR_{cysC}$ decline of -1.17 (-1.31; -1.03) and -2.07 (-2.37; -1.78) mL/min/1.73m^2 [difference -0.90 (-1.23; -0.57)] (Table 2). Patients with normal or high blood pressure had annual decline rates of -1.01 (-1.20; -0.82) and -1.51 (-1.69; -1.34) mL/min/1.73m^2 [difference -0.50 (-0.76; -0.24)]. Successive quartiles of systolic blood pressure (quartile ranges: 86.5 to 128.0; 128.5 to141.5; 142.0 to 156.5; 157.0 to 237.5 mmHg) showed a faster annual kidney function decline: -0.99 (-1.25; -0.74), -1.10 (-1.34; -0.85), -1.45 (-1.70; -1.20) and -1.82 (-2.07; -1.57) mL/min/1.73m^2, respectively. Each 10 mmHg systolic blood pressure increment was associated with an extra annual kidney function decline of -0.17 (-0.23; -0.12, P<0.001) mL/min/1.73m^2. We found a weak U-shaped relation between diastolic blood pressure and kidney function decline. Annual kidney function decline for patients in the lowest through the highest quartile was -1.33 (-1.59; -1.08), -1.15 (-1.40; -0.91), -1.32 (-1.58; -1.06) and -1.55 (-1.80; -1.30) mL/min/1.73m^2 (quartile ranges: 44.0 to 73.5; 74.0 to 81.0; 81.5 to 88.0; 88.5 to 124.0 mmHg). We found no significant difference in the rate of annual kidney function decline between patients with high compared to normal LDL levels. Smokers of cigarettes compared to non-smokers had an additional annual $eGFR_{cysC}$ decline of -0.19 (-0.54; 0.16) mL/min/1.73m^2. Obesity was associated with a 23% faster kidney function decline (Table 2). We found no evidence for effect modification between sex, age, strata of baseline kidney function and the pre-specified cardiovascular risk factors with regard to $eGFR_{cysC}$ decline.

Of all patients, 573 (24.4%) had a rapid kidney function decline. Table 3 shows the odds ratios (OR) for rapid kidney function decline according the a priori selected cardiovascular risk factors. Especially, diabetes [OR 1.72 (1.36; 2.17)] and high blood pressure [OR 1.43 (1.18; 1.74)] were strongly associated with rapid kidney function decline. Associations for current smoking [OR 1.21 (0.94; 1.57)] and obesity [OR 1.15 (0.92; 1.45)] were weaker. High LDL was associated with slower kidney function decline [OR 0.80 (0.66; 0.98)]. Results were comparable when defining rapid kidney function decline as >5 mL/min/1.73m^2 per year (data not shown). Furthermore, after adjustment for age and treatment group, men had a slightly higher risk on rapid kidney function decline than women [OR 1.13 (0.89; 1.43)]. After adjustment for sex and treatment group, older compared to younger patients had more often rapid kidney function decline [age ≥70y *vs* <70y OR 1.32 (1.09; 1.59), age ≥75y *vs* <75y OR 1.59 (1.25; 2.02)].

Table 2: Mean (95%-CI) annual cystatin C-based eGFR decline rates in 2426 post-MI patients according to absence or presence of cardiovascular risk factors.

Risk factor		n	Crude	Model 1	Model 2
Diabetes	No (ref)	1977	-1.17 (-1.31; -1.03)	-1.17 (-1.31; -1.03)	-1.17 (-1.31; -1.03)
	Yes	449	-2.06 (-2.36; -1.77)**	-2.10 (-2.40; -1.81)**	-2.07 (-2.37; -1.78)**
Blood pressure	<140/90 mmHg (ref)	1062	-1.01 (-1.20; -0.82)	-1.01 (-1.20; -0.82)	-1.01 (-1.20; -0.82)
	≥140/90 mmHg	1364	-1.59 (-1.76; -1.42)**	-1.51 (-1.69; -1.34)**	-1.51 (-1.69; -1.34)**
Serum LDL	<2.5 mmol/L (ref)	990	-1.47 (-1.67; -1.27)	-1.47 (-1.67; -1.27)	-1.47 (-1.67; -1.27)
	≥2.5 mmol/L	1436	-1.25 (-1.41; -1.08)	-1.29 (-1.46; -1.12)	-1.28 (-1.45; -1.12)
Cigarette smoking	Non-smoking (ref)	2040	-1.32 (-1.46; -1.18)	-1.32 (-1.46; -1.18)	-1.32 (-1.46; -1.18)
	Currently smoking	386	-1.43 (-1.75; -1.11)	-1.54 (-1.86; -1.22)	-1.51 (-1.83; -1.18)
Body mass index	<30.0 kg/m^2 (ref)	1871	-1.30 (-1.45; -1.16)	-1.30 (-1.45; -1.16)	-1.30 (-1.45; -1.16)
	≥30.0 kg/m^2	555	-1.46 (-1.72; -1.19)	-1.62 (-1.89; -1.35)*	-1.61 (-1.88; -1.34)

CI, confidence interval; LDL, low-density lipoprotein.
Adjusted variables were fixed at the mean value of the reference group, hence the estimates of the reference category are equal across models. Diabetes was defined as self-reported diagnosis by a physician, use of glucose-lowering drugs, or in case of elevated plasma glucose level
* p<0.05, ** p<0.001 for difference between presence vs absence of risk factor.
Model 1: adjusted for age, sex and treatment group.
Model 2: Model 1 plus additional adjustment for current smoking, alcohol consumption, level of education, diabetes, high blood pressure, high LDL, and obesity. Analyses for obesity were not adjusted for diabetes, high blood pressure, and high LDL.

Table 3: Odds ratios (95%-CI) for risk of rapid $eGFR_{cysC}$ decline (>3 mL/min/1.73m² per year) in 2426 post-MI patients, for different cardiovascular risk factors.

Risk factor¶	Crude	Model 1	Model 2
Diabetes	1.77 (1.41; 2.21)**	1.79 (1.43; 2.25)**	1.72 (1.36; 2.17)**
High blood pressure	1.48 (1.22; 1.79)**	1.41 (1.17; 1.72)**	1.43 (1.18; 1.74)**
High LDL	0.81 (0.67; 0.98)*	0.82 (0.68; 0.99)*	0.80 (0.66; 0.98)*
Current cigarette smoking	1.13 (0.88; 1.45)	1.23 (0.95; 1.58)	1.21 (0.94; 1.57)
Obesity	1.09 (0.88; 1.36)	1.17 (0.93; 1.46)	1.15 (0.92; 1.45)

CI, confidence interval; LDL, low-density lipoprotein.
* p<0.05, ** p<0.001
¶ Reference: absence of the risk factor of interest.
Diabetes was defined as self-reported diagnosis by a physician, use of glucose-lowering drugs, or hyperglycemia. High blood pressure was defined as systolic blood pressure of ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg, irrespective of use of blood pressure lowering drugs. High LDL was defined as serum LDL level ≥2.5 mmol/L. Obesity was defined as BMI ≥30.0 kg/m².
Model 1: adjusted for age, sex and treatment group.
Model 2: Model 1 plus additional adjustment for current smoking, alcohol consumption, level of education, diabetes, high blood pressure, high LDL, and obesity. Analyses for obesity were not adjusted for diabetes, high blood pressure, and high LDL.

Kidney function decline and number of risk factors

We calculated the annual kidney function decline and risk for rapid kidney function decline in each patient according to the number of cardiovascular risk factors; diabetes, high blood pressure, current smoking of cigarettes and obesity (BMI ≥ 30kg/m²). Patients without any of these cardiovascular risk factors present (thus with optimal cardiovascular parameters and healthy lifestyle) had an annual kidney function decline of -0.90 (-1.16; -0.65) mL/min/1.73m² (Table 4, Figure 1). Each additional cardiovascular risk factor was associated with a progressively faster annual kidney function decline [linear regression coefficient -0.45 (-0.59; -0.30) per additional risk factor, P for linear trend <0.001)]. Patients in whom three or more cardiovascular risk factors were present had an annual kidney function decline of -2.37 (-2.85; -1.89) mL/min/1.73m². Risk for rapid kidney function decline increased progressively with every additional cardiovascular risk factor (Table 4, Figure 1). Patients with at least three cardiovascular risk factors had a 2.5-fold increased risk compared to patients without any cardiovascular risk factor. Patients in whom all four cardiovascular risk factors (n=16) were present, had an annual kidney function decline of -4.59 (-5.38; -3.79) mL/min/1.73m².

Table 4: Mean (95%-CI) annual $eGFR_{cysC}$ decline and odds ratios (95%-CI) for rapid $eGFR_{cysC}$ decline (>3 mL/min/1.73m² per year) are presented per number of cardiovascular risk factors¶, in 2426 post-MI patients of the Alpha Omega cohort.

Number of risk factors¶	n		Crude	Model 1	Model 2
0 (ref)	597	Annual $eGFR_{cysC}$ decline	-0.90 (-1.16; -0.65)	-0.90 (-1.16; -0.65)	-0.90 (-1.16; -0.65)
1	1088		-1.29 (-1.48; -1.10)	-1.23 (-1.42; -1.03)	-1.23 (-1.42; -1.03)
2	574		-1.61 (-1.87; -1.35)	-1.66 (-1.92; -1.40)	-1.65 (-1.91; -1.39)
≥3	167		-2.26 (-2.74; -1.77)	-2.37 (-2.85; -1.88)	-2.37 (-2.85; -1.89)
0 (ref)	597	OR rapid decline	1	1	1
1	1088		1.14 (0.89; 1.46)	1.09 (0.85; 1.40)	1.09 (0.85; 1.40)
2	574		1.55 (1.18; 2.03)	1.57 (1.19; 2.06)	1.56 (1.19; 2.06)
≥3	167		2.41 (1.66; 3.49)	2.55 (1.77; 3.71)	2.55 (1.75; 3.71)

CI, confidence interval; OR, Odds ratio.
Adjusted variables were fixed at the mean value of the reference group, hence the estimates of the reference category are equal across models.
¶ Cardiovascular risk factors included: diabetes, high blood pressure, obesity, current smoking.
Diabetes was defined as self-reported diagnosis by a physician, use of glucose-lowering drugs, or hyperglycemia. High blood pressure was defined as systolic blood pressure of ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg, irrespective of use of blood pressure lowering drugs. Obesity was defined as BMI ≥30.0 kg/m².
Model 1: adjusted for age, sex and treatment group.
Model 2: Model 1 plus additional adjustment for alcohol consumption and level of education.

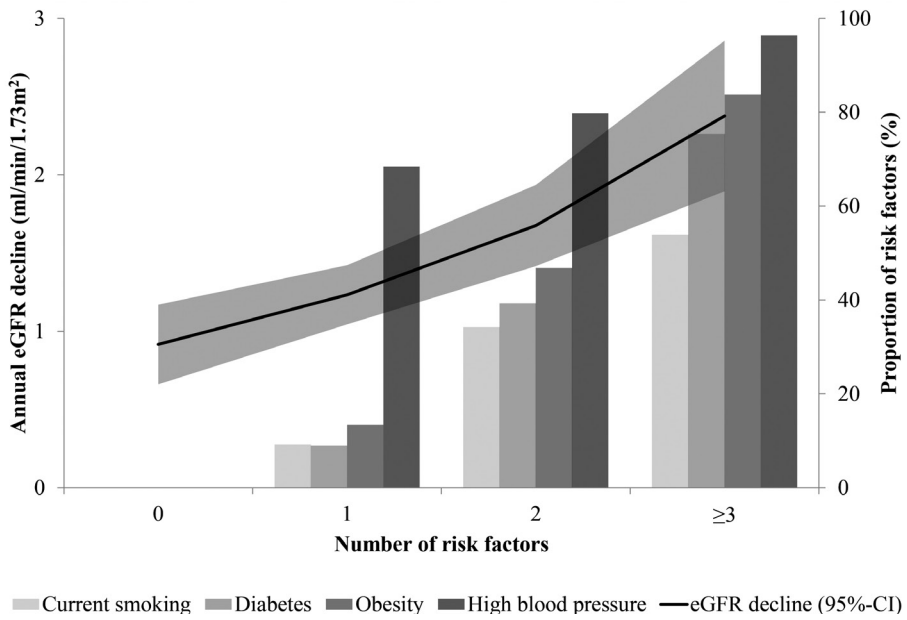

Figure 1: Annual $eGFR_{cysC}$ decline according to the number of cardiovascular risk factors, in 2426 post-MI patients in the Alpha Omega cohort. For the four groups according to the number of cardiovascular risk factors (diabetes, high blood pressure, obesity, and current smoking) the proportion (%) of the four different risk factors (columns, right vertical axis) and the mean (95%-CI) annual $eGFR_{cysC}$ decline (black line, left vertical axis), adjusted for age, sex, and treatment group are presented.

SENSITIVITY ANALYSES

Results did not materially change when using complete cases only, instead of multiple imputed data. Adjustment with continuous instead of dichotomized variables did not change the results. Adjustment for time since MI, serum hsCRP, or use of RAS blocking drugs yielded similar results. Confining analyses to patients who persistently used RAS blocking drugs (n=1206), yielded comparable associations. Analyses based on $eGFR_{cr\text{-}cysC}$ as outcome showed slightly weaker effect estimates (Supplementary Table S1 and S2). The association between the number of cardiovascular risk factors and annual $eGFR_{cr\text{-}cysC}$ or $eGFR_{cysC}$ decline was comparable (Supplementary Table S3).

DISCUSSION

We showed in a cohort of stable older post-MI patients, that those patients with optimally treated cardiovascular risk factors, including healthy lifestyle, had an annual kidney function decline of about -0.90 mL/min/1.73m^2. In contrast, post-MI patients with three or more suboptimal treated cardiovascular risk factors, had a 2.5-fold faster annual kidney function decline of about -2.37 mL/min/1.73m^2. We recently showed that in these post-MI patients, an $eGFR_{cysC}$ below 80 mL/min/1.73m^2 is a graded risk factor for cardiovascular and all-cause mortality, underlining the clinical relevance of these findings.[2, 28]

The mean annual kidney function decline of -0.90 (-1.16; -0.65) mL/min/1.73m^2 that we found in optimally treated cardiac patients with healthy lifestyle, is within the normal range of the age-related kidney function decline in healthy individuals of -1.0 mL/min/1.73m^2.[32-34] The mean (95%-CI) annual kidney function decline of all patients was -1.34 (-1.47; -1.21) mL/min/1.73m^2. Previously, the PREVEND study reported in post-MI patients an annual kidney function decline (95%-CI) of -2.2 (-5.0; -0.9) mL/min/1.73m^2.[5] Post-MI patients of the PREVEND study had a similar cardiovascular risk profile compared to patients of the Alpha Omega cohort. There are several explanations that may have resulted in the higher annual kidney function decline in the PREVEND compared to the Alpha Omega cohort. First, the small number (n=66) of post-MI patients in the PREVEND study resulted in a wide 95%-confidence interval and as a consequence the effect-estimate is less precise. Patients from the Alpha Omega cohort participated in a trial for 41 months. Trial patients generally are healthier and more compliant compared to the general population, a phenomenon known as volunteer bias.[35] Finally, during follow-up patients in the Alpha Omega cohort (2002 to 2009) have been more strictly controlled according to more recent secondary prevention guidelines, compared to patients in the PREVEND cohort (1997 to 2005). In updated guidelines there was especially more attention for patient education, lifestyle monitoring (e.g. smoking cessation, weight management), diabetes and high blood pressure management, more strict lipid regulation and standard prescription of statins and ACE-inhibitors.[36, 37]

In agreement with other studies, we found that diabetes and high blood pressure were strongly associated with accelerated kidney function decline.[7-10] Diabetes may lead to diabetic nephropathy; a complex disease characterized by hemodynamic, metabolic, and inflammatory changes, ultimately leading to progressive interstitial fibrosis and glomerular damage.[38] High blood pressure may cause increased intraglomerular pressure, leading to endothelial dysfunction, loss of adequate auto-regulation and eventually to progressive glomerular and interstitial fibrosis.[39] We found a weak association between high LDL level and slower kidney function decline, but this association may be distorted by the fact that 85% of all patients used statins.

Moreover, of all patients with high LDL at baseline, 10% started with a statin during follow-up. Our finding is in concordance with recent guidelines, stating that CKD patients ≥50y should be treated with a statin independent of lipid levels without trying to reach a target level.[40] This paradigm shift is caused by the lack of evidence linking changes in lipid levels with actual cardiovascular risk and emerging evidence showing pleiotropic effects of statins.[30, 41, 42]

In line with other studies, we found that obesity was associated with faster kidney function decline.[8, 43] Obesity promotes deterioration of kidney function through cardiovascular risk factors, such as diabetes and hypertension, and is also associated with visceral fat accumulation and accompanying inflammation, leading to glomerular and interstitial fibrosis.[12, 13, 24] Furthermore, we found that smoking of cigarettes was associated with kidney function decline, which is confirmed by other studies. [44] However, the association of smoking and kidney function decline was weaker than expected; and could be underestimated due to underreporting, so called information bias.

Our study has some limitations. First, the observational design prevents us from making causal inferences. Second, kidney function was estimated at only two time points and was not directly measured. Third, we had no information about proteinuria, an important independent predictor of kidney function. Therefore, we could not study the association between optimal treatment of cardiovascular risk factors and change of proteinuria. Fourth, about 17% of patients dropped out owing to missing samples, refused participation, or death. If anything, this may have resulted in underestimation of the associations that we found, since patients who dropped-out were most likely less healthy. Fifth, volunteer bias may be present, since we only included trial patients. However, since volunteering patients usually are more healthy, we expect this may have led to an underestimation of our results. Finally, we analyzed post-MI patients only, which may hamper generalizability of our results. Notably, the prevalence of cardiovascular disease shows an increasing trend worldwide, our cohort of patients therefore represents a growing patient group.

A major strength of this study is our large homogeneous population of post-MI patients, which provides a unique opportunity to study the course of kidney function decline in these patients. Second, we used both $eGFR_{cysC}$ and $eGFR_{cr\text{-}cysC}$ as outcome, currently the most accurate available methods to estimate kidney function.[26, 28, 45]

To conclude, we found a faster rate of kidney function decline in post-MI patients with an increasing number of insufficiently treated cardiovascular risk factors (including unhealthy lifestyle). Post-MI patients with optimal cardiovascular and lifestyle parameters have an annual kidney function decline comparable to the general population. Further research is needed to investigate whether optimization of cardiovascular risk factors and healthy lifestyle may slow down the accelerated kidney function decline in post-MI patients.

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The Alpha Omega Cohort Study is registered at ClinicalTrials.gov (no. NCT03192410). Results from this study have been previously presented at the ERA-EDTA Congress in Madrid, June 2017 (poster 55-SP) and at the Dutch Nephrology Days (NND) March 2017.

DISCLOSURES

EH is a member of the Guideline Committee of the Dutch Federation of Nephrology. JG received research funding from Unilever R&D for epidemiological studies of dietary fatty acids and is a member of the Standing Committee on Nutrition of the Dutch Health Council, Working Group on Minerals of the European Food and Safety Authority, and Dutch Academy for Nutritional Sciences, and is a Fellow of the American Heart Association. DK received research funding from the Royal Netherlands Academy of Arts and Sciences and is Member of the Dutch Academy of Nutritional Sciences. KE, JF, and EG report that they have no disclosures.

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AUTHORS' CONTRIBUTIONS

KE, JG, DK and EH contributed to conception and design of the manuscript. KE, JG, EG, DK and EH contributed to acquisition, analysis and interpretation, and drafted the manuscript. JF contributed to interpretation. All authors critically revised the manuscript, all gave final approval and all agreed to be accountable for all aspects of work, ensuring integrity and accuracy.

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SUPPLEMENTARY DATA

Table S1: Mean (95%-CI) annual creatinine cystatin C-based eGFR decline rates in 2344 post-MI patients according to absence or presence of cardiovascular risk factors.

Risk factor		n	Crude	Model 1	Model 2
Diabetes	No (ref)	1906	-1.59 (-1.75; -1.42)	-1.59 (-1.75; -1.42)	-1.59 (-1.75; -1.42)
	Yes	438	-2.38 (-2.73; -2.03)*	-2.36 (-2.71; -2.01)*	-2.34 (-2.69; -1.98)*
Blood pressure	<140/90 mmHg (ref)	1026	-1.43 (-1.66; -1.20)	-1.43 (-1.66; -1.20)	-1.43 (-1.66; -1.20)
	≥140/90 mmHg	1318	-1.97 (-2.18; -1.77)*	-1.94 (-2.14; -1.73)*	-1.95 (-2.15; -1.74)*
Serum LDL	<2.5 mmol/L (ref)	957	-1.85 (-2.09; -1.61)	-1.85 (-2.09; -1.61)	-1.85 (-2.09; -1.61)
	≥2.5 mmol/L	1387	-1.66 (-1.85; -1.46)	-1.66 (-1.86; -1.46)	-1.66 (-1.86; -1.46)
Cigarette smoking	Non-smoking (ref)	1983	-1.73 (-1.89; -1.56)	-1.73 (-1.89; -1.56)	-1.73 (-1.89; -1.56)
	Currently smoking	361	-1.77 (-2.15; -1.38)	-1.83 (-2.22; -1.44)	-1.80 (-2.19; -1.41)
Body mass index	<30.0 kg/m^2 (ref)	1814	-1.68 (-1.86; -1.51)	-1.68 (-1.86; -1.51)	-1.68 (-1.86; -1.51)
	≥30.0 kg/m^2	530	-1.91 (-2.23; -1.59)	-1.95 (-2.27; -1.62)	-1.93 (-2.26; -1.61)

CI, confidence interval; LDL, low-density lipoprotein.
Adjusted variables were fixed at the mean value of the reference group, hence the estimates of the reference category are equal across models. Diabetes was defined as self-reported diagnosis by a physician, use of glucose-lowering drugs, or hyperglycemia.
* p<0.001 for difference between presence *vs* absence of risk factor.
Model 1: adjusted for age, sex and treatment group.
Model 2: Model 1 plus additional adjustment for current smoking, alcohol consumption, level of education, diabetes, high blood pressure, high LDL, and obesity. Analyses for obesity were not adjusted for diabetes, high blood pressure, and high LDL.

Table S2: Odds ratios (95%-CI) for risk of rapid creatinine cystatin C-based eGFR decline (>3 mL/min/1.73m² per year) in 2344 post-MI patients, for different cardiovascular risk factors.

Risk factor ¶	Crude	Model 1	Model 2
Diabetes	1.28 (1.04; 1.59)*	1.27 (1.02; 1.58)*	1.24 (0.99; 1.54)
High blood pressure	1.30 (1.09; 1.54)*	1.28 (1.07; 1.52)*	1.31 (1.10; 1.56)*
High LDL	0.81 (0.68; 0.96)*	0.81 (0.68; 0.96)*	0.80 (0.67; 0.95)*
Current cigarette smoking	0.90 (0.71; 1.14)	0.93 (0.73; 1.18)	0.92 (0.72; 1.17)
Obesity	1.07 (0.87; 1.31)	1.06 (0.86; 1.31)	1.05 (0.85; 1.29)

CI, confidence interval; LDL, low-density lipoprotein, eGFR, estimated glomerular filtration rate.
* p<0.05
¶ Reference: absence of the risk factor of interest.
Diabetes was defined as self-reported diagnosis by a physician, use of glucose-lowering drugs, or hyperglycemia. High blood pressure was defined as systolic blood pressure of ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg, irrespective of use of blood pressure lowering drugs. High LDL was defined as serum LDL level ≥2.5 mmol/L. Obesity was defined as BMI ≥30.0 kg/m².
Model 1: adjusted for age, sex and treatment group.
Model 2: Model 1 plus additional adjustment for current smoking, alcohol consumption, level of education, diabetes, high blood pressure, high LDL, and obesity. Analyses for obesity were not adjusted for diabetes, high blood pressure, and high LDL.

Table S3: Mean (95%-CI) annual creatinine cystatin C-based eGFR decline and odds ratios (95%-CI) for rapid creatinine cystatin C-based eGFR decline (>3 mL/min/1.73m² per year) per number of cardiovascular risk factors¶, in 2344 post-myocardial infarction patients of the Alpha Omega cohort.

Number of risk factors¶	n		Crude	Model 1	Model 2
0 (ref)	581	Annual $eGFR_{cr-cysC}$	-1.33 (-1.63; -1.02)	-1.33 (-1.63; -1.02)	-1.33 (-1.63; -1.02)
1	1051	decline	-1.72 (-1.95; -1.50)	-1.69 (-1.92; -1.46)	-1.69 (-1.92; -1.46)
2	554		-1.88 (-2.19; -1.57)	-1.87 (-2.18; -1.56)	-1.87 (-2.18; -1.55)
≥3	158		-2.83 (-3.41; -2.24)	-2.84 (-3.43; -2.26)	-2.85 (-3.44; -2.27)
0 (ref)	581	OR rapid decline	1	1	1
1	1051		1.03 (0.83; 1.28)	1.01 (0.81; 1.25)	1.00 (0.81; 1.25)
2	554		1.19 (0.93; 1.52)	1.17 (0.91; 1.50)	1.16 (0.90; 1.48)
≥3	158		1.57 (1.09; 2.25)	1.56 (1.08; 2.24)	1.56 (1.08; 2.24)

CI, confidence interval; OR, Odds ratio; eGFR, estimated glomerular filtration rate.
Adjusted variables were fixed at the mean value of the reference group, hence the estimates of the reference category are equal across models.
¶ Cardiovascular risk factors included: diabetes, high blood pressure, obesity, current smoking.
Diabetes was defined as self-reported diagnosis by a physician, use of glucose-lowering drugs, or hyperglycemia. High blood pressure was defined as systolic blood pressure of ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg, irrespective of use of blood pressure lowering drugs. Obesity was defined as BMI ≥30.0 kg/m².
Model 1: adjusted for age, sex and treatment group.
Model 2: Model 1 plus additional adjustment for alcohol consumption and level of education

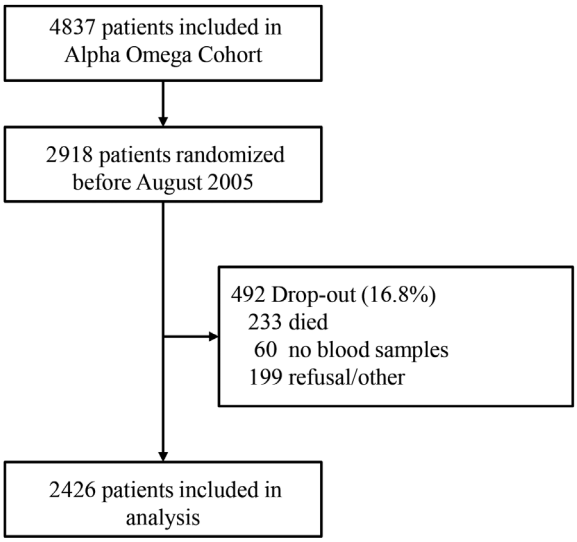

Figure S1: Flow chart of 2426 post-MI patients included in the present study. The patients randomized before August 2005 are considered a random sample of the total population of 4837 patients. Of all deceased patients, 3 died of renal failure.

Chapter 3 –

Body-fat indicators and kidney function decline in older post-myocardial infarction patients: The Alpha Omega Cohort study

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ABSTRACT

Background: Obesity increases risk of hypertension and diabetes, the leading causes of end-stage renal disease. The effect of obesity on kidney function decline in stable post-myocardial infarction (MI) patients is poorly documented. This relation was investigated in a large cohort of older post-MI patients.

Design: Data were analyzed from 2410 post-MI patients in the Alpha Omega Trial, aged 60–80 years receiving optimal pharmacotherapy treatment (79% men, 18% diabetes).

Methods: Cystatin C based estimated glomerular filtration rate ($eGFR_{cysC}$) was calculated at baseline and after 41 months, using the CKD-EPI equation. Obesity was defined as body-mass index (BMI) ≥30 kg/m^2 and high waist circumference (WC) as ≥102 and ≥88 cm for men and women. The relation between BMI, WC and annual $eGFR_{cysC}$ decline was evaluated by linear regression.

Results: At baseline, mean (SD) $eGFR_{cysC}$ was 81.5 (19.6) ml/min/1.73m^2, 23% of all patients were obese. After multivariable adjustment, the annual mean (95%-CI) $eGFR_{cysC}$ decline in men and women was -1.45 (-1.59 to -1.31) and -0.92 (-1.20 to -0.63) ml/min/1.73m^2, respectively (P=0.001). Obese versus non-obese patients and patients with high versus normal WC experienced greater annual $eGFR_{cysC}$ decline. Men and women showed an additional annual $eGFR_{cysC}$ decline of -0.35 (-0.56 to -0.14) and -0.21 (-0.55 to 0.14) ml/min/1.73m^2 per 5 kg/m^2 BMI increment (P for interaction 0.3).

Conclusions: High compared to normal BMI or WC were associated with more rapid kidney function decline in older stable post-MI patients receiving optimal drug therapy.

INTRODUCTION

The prevalence of obesity has increased to epidemic proportions and is ranked globally in the top five risk factors for death.[1] Obesity, defined as a body mass index (BMI) of ≥30 kg/m^2, is associated with an increased risk of cardiovascular morbidity and mortality, as well as accelerated kidney function decline.[1–3] Impaired kidney function itself is a robust and independent risk factor for cardiovascular morbidity and mortality.[4] The annual rate of kidney function decline in post-myocardial infarction (MI) patients is more than double that of the general population.[5, 6]

Obesity may promote kidney damage through both hemodynamic and hormonal effects. The deleterious effects of obesity on the kidney are, in part, mediated by cardiovascular risk factors such as diabetes mellitus, hypertension and dyslipidemia.[1]Additionally, accumulation of visceral fat can increase production of inflammatory mediators by adipocytes, contributing to glomerular and interstitial fibrosis.[7] Furthermore, obesity is associated with an increase in the single-nephron glomerular filtration rate, which may lead to glomerulosclerosis and subsequent progressive loss of kidney function.[8]

Several studies have suggested a paradoxical effect of obesity in individuals with pre-existing chronic illness, such as chronic kidney disease, showing that obesity is associated with improved survival or kidney function.[9, 10] This "obesity paradox" challenges current guidelines, which advise weight reduction towards an ideal BMI of 20–25 kg/m^2.[11]

The aim of this study was to assess the association between obesity and the rate of kidney function decline in older post-MI patients receiving state-of-the-art drug treatment, separately for men and women, who differ in body composition. These results might inform care guidelines for post-MI patients.

METHODS

Study design

This is a secondary analysis of the prospective Alpha Omega Cohort study (ClinicalTrials.gov no. NCT03192410). The cohort consists of patients included in the Alpha Omega Trial, a randomized controlled trial of omega-3 (n-3) fatty acid supplementation undertaken in 4837 patients aged 60-80 years with a verified history of MI. Patients received state-of-the-art antihypertensive, antithrombotic and lipid-modifying drug treatment, as described in detail elsewhere.[12] The trial started in 2002 and ended in 2009. For this study, patients were selected from whom non-fasting blood was drawn at baseline and after

41 months. Owing to financial constraints, two blood samples were obtained in only 2426 patients (50% of the cohort), i.e. those randomized before August 2005. Of all patients randomized prior to August 2005 (n=2918), 233 patients died during follow-up, 259 patients had missing blood samples or declined to participate, and 16 patients had missing data on BMI and/or waist circumference (WC), yielding an evaluable cohort of 2410 patients (Supplementary Figure S1). The study was conducted in accordance with the Helsinki Declaration and was approved by a central and local medical ethics committee in the Netherlands. Written informed consent was obtained from all patients.

Body mass index and waist circumference

Body weight and height were measured with the subject wearing light indoor clothing without shoes. BMI was calculated as weight in kilograms divided by the square of height in meters. Following World Health Organization (WHO) guidelines, normal weight was defined as a BMI of 18.5–24.9 kg/m^2, overweight as a BMI of 25.0–29.9 kg/m^2 and obesity as a BMI of 30.0 kg/m^2 or greater.[1] WC, measured at the midpoint between the bottom rib and the top of the hipbone, was used as a proxy of visceral fat. Men with a WC ≥102 cm and women with a WC ≥88 cm were considered to have a high risk of metabolic complications, hereafter referred to as high, as opposed to normal, WC.[1]

Kidney function assessment

At baseline and after 41 months follow-up we measured from stored blood serum cystatin C (cysC) using a particle-enhanced immunonephelometric assay and serum creatinine (cr) by the modified kinetic Jaffé method, as previously described in detail.[4] We estimated glomerular filtration rate (eGFR) with cysC alone and the combined cr-cysC Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations from 2012, taking into account age, sex and race.[13] In the main analyses results are shown for $eGFR_{cysC}$, as it is recommended for confirmatory testing in the current KDIGO guidelines.[11] In the supplements the results are presented for $eGFR_{cr\text{-}cysC}$. The change (or slope) of the $eGFR_{cysC}$ and $eGFR_{cr\text{-}cysC}$ from baseline to 41 months was calculated for each patient by subtracting the eGFR at baseline from the eGFR after 41 months. Assuming a linear kidney function decline during follow-up, we then calculated an annual decline rate. Rapid kidney function decline was defined as an annual $eGFR_{cysC}$ decline of ≥3 mL/min/1.73m^2.[14]

Data collection

Patients were interviewed and physically examined by trained research nurses at home or in the hospital at baseline and after 41 months. Lipid, glucose and high-sensitivity C-reactive protein (hsCRP) levels were determined as described elsewhere.[15] Information on demographic variables, lifestyle habits, current health status, and medical history were collected by self-administered questionnaires, as previously described.[12] Questionnaires were checked by research nurses. Diabetes mellitus was considered present in case of a self-reported physician diagnosis, use of glucose-lowering drugs, and/or elevated blood glucose. We used the average of two blood pressure measurements after a 10 min seated rest. Medication was coded according to the Anatomical Therapeutic Chemical (ATC) Classification System.

Data analysis

Baseline characteristics are presented as mean (SD), median (interquartile range), or number (percentage) as appropriate. Missing data on level of education (n=14) were imputed by the sex-specific mode. The relation between BMI or WC and kidney function decline met the linear regression assumptions. ANCOVA was used to calculate mean annual eGFR decline rates per WHO category of BMI and for high and normal WC. Normal BMI or normal WC was applied as the reference category. In these analyses, 2 patients with a BMI <18.5 kg/m^2 were excluded. Linear regression was used to study the association between BMI or WC as continuous variables and kidney function decline. Regression coefficients were calculated per 5 kg/m^2 increment of BMI (approximately 1 SD), corresponding to the width of each WHO category; and per 10 cm increment of WC, which approximately corresponds to a 5 kg/m^2 increment of BMI.[16]

The continuous relation between each indicator of body fat (BMI and WC) and kidney function decline was further analyzed in a flexible manner using four-knot restricted cubic splines with 95% confidence intervals (CIs). As per general guidelines, the knots were chosen at the 5th, 35th, 65th and 95th percentile of the BMI and WC distribution for men and women separately.[17]

All analyses were adjusted for the n-3 fatty acid treatment groups of the Alpha Omega Trial (three dummy variables). In addition to the treatment group, we adjusted for age at baseline and sex (model 1). According to the WHO, smoking of cigarettes, alcohol consumption and socio-economic status may confound the association of obesity with outcome.[1] Therefore, in model 2 (full model), an additional adjustment was made for these baseline factors: current cigarette smoking (yes, no), alcohol use (yes, no), and level of education (elementary education, low, intermediate and high education) as a proxy for socio-economic status. Analyses were not adjusted for baseline eGFR, since baseline-adjustment

in models with change-scores as outcome variable results in biased estimates.[18] In the main analyses we did not control for variables considered likely causal intermediates in the relation between obesity and kidney function decline, such as blood pressure, diabetes, and low-density lipoprotein (LDL)-cholesterol.

Sensitivity analyses

Several sensitivity analyses were performed. First, we included factors in the causal pathway, diabetes, systolic blood pressure and LDL-cholesterol, to estimate the presence of mediation. In a separate analysis we controlled for use of renin-angiotensin system (RAS) blocking drugs and physical activity. We explored the presence of effect measure modification between treatment group and BMI or WC with regard to kidney function decline. Finally, we investigated the potential relation between change in BMI or WC from baseline to 41 months follow-up and annual eGFR decline. The main analyses were repeated using $eGFR_{cr-cysC}$ decline as outcome. [14]All results are presented for men and women separately, given previously reported differences in kidney function decline between men and women.

Two-sided P-values <0.05 were considered statistically significant. All analyses were performed using SPSS 23.0 (SPSS, Inc., Chicago, IL, USA) and STATA Statistical Software (Statacorp, College Station, TX, USA), version 14.1.

RESULTS

Baseline characteristics

Of all patients, mean age was 69 years, 79% were men, and 99% were white, median time since MI was 4.0 years. Baseline characteristics according to BMI categories (normal weight, overweight, and obesity) are presented in Table 1. Patients with overweight or obesity compared to normal weight had more often diabetes, used more often blood pressure lowering drugs, had higher serum cholesterol levels, higher hsCRP levels and lower baseline $eGFR_{cysC}$. A similar trend was observed when comparing low and high WC categories (Supplementary Table S1). Mean (SD) baseline BMI was 27.5 (3.3) kg/m^2 for men and 28.4 (4.6) kg/m^2 for women. Mean (SD) WC at baseline was 102 (9) cm for men and 97 (12) cm for women. Women compared to men had more often diabetes, used more often blood pressure lowering drugs (Supplementary Table S2). BMI and WC were strongly correlated (Pearson correlation coefficient 0.8). Each 1 kg/m^2 increment of BMI was associated with an additional 2.2 (95% CI 2.1 to 2.3) cm increment of WC.

Table 1: Baseline characteristics of 2408 post-myocardial infarction patients, stratified by three categories of weight status according to the WHO classification.

	Normal weight (n=527)	Overweight (n=1328)	Obese (n=553)
Age, years	69.3 ± 5.4	69.0 ± 5.4	68.0 ± 5.5
Men, no (%)	419 (79.5)	1116 (84.0)	379 (68.5)
Ethnicity, white, no. (%)	522 (99.1)	1310 (98.6)	548 (99.1)
Higher education,[a] n (%)	77 (14.6)	171 (12.9)	49 (8.9)
Current smoking, no. (%)	106 (20.1)	188 (14.2)	89 (16.1)
Alcohol use,[b] n (%)	388 (73.6)	1004 (75.6)	352 (63.7)
Height, cm	172.5 ± 7.9	173.1 ± 7.8	170.0 ± 8.8
Weight, kg	69.6 ± 7.8	82.0 ± 8.3	94.7 ± 11.7
Body mass index, kg/m^2	23.3 ± 1.4	27.3 ± 1.4	32.7 ± 2.7
Waist circumference, cm	91.6 ± 7.3	100.9 ± 6.7	111.3 ± 9.2
Physically active,[c] n (%)	119 (22.6)	319 (24.0)	92 (16.6)
Time since myocardial infarction, yr	3.6 (1.6–6.1)	4.0 (2.0–6.3)	4.5 (2.4–6.9)
Diabetes,[d] n (%)	65 (12.3)	217 (16.3)	162 (29.3)
Systolic blood pressure, mmHg	141.3 ± 21.8	144.3 ± 21.4	142.8 ± 20.7
Diastolic blood pressure, mmHg	79.4 ± 10.6	82.0 ± 10.7	82.0 ± 10.5
Antihypertensive drugs,[e] n (%)	449 (85.2)	1141 (85.9)	505 (91.3)
ACE inhibitors/ATII blockers	265 (50.3)	704 (53.0)	330 (59.7)
Beta blockers	324 (61.5)	863 (65.0)	386 (69.8)
Calcium channel blockers	97 (18.4)	248 (18.7)	117 (21.2)
Diuretics	78 (14.8)	242 (18.2)	177 (32.0)
Glucose-lowering drugs,[f] n (%)	48 (9.1)	159 (12.0)	108 (19.5)
Insulin analogues	11 (2.1)	42 (3.2)	51 (9.2)
Oral glucose-lowering drugs	39 (7.4)	131 (9.9)	81 (14.6)
Lipid-modifying drugs,[g] n (%)	454 (86.1)	1140 (85.8)	480 (86.8)
Statins	452 (85.8)	1129 (85.0)	477 (86.3)
Antithrombotic agents,[h] n (%)	516 (97.9)	1294 (97.4)	541 (97.8)
Total cholesterol,[i] mmol/L	4.78 ± 0.92	4.81 ± 0.94	4.95 ± 0.91
HDL,[i] mmol/L	1.35 ± 0.36	1.25 ± 0.31	1.19 ± 0.31
LDL,[i] mmol/L	2.72 ± 0.79	2.74 ± 0.80	2.77 ± 0.80
Triglycerides,[j] mmol/L	1.41 (1.04–1.91)	1.62 (1.21–2.24)	1.96 (1.51–2.73)

Table 1: Continued

	Normal weight (n=527)	Overweight (n=1328)	Obese (n=553)
Plasma glucose,[k] mmol/L	5.6 ± 1.7	5.9 ± 1.8	6.6 ± 2.4
High-sensitivity CRP, mg/L	1.24 (0.62–2.73)	1.58 (0.81–3.37)	2.60 (1.11–4.81)
Serum cystatin C, mg/L	0.96 ± 0.23	0.96 ± 0.24	1.00 ± 0.26
Serum creatinine,[l] µmol/L	88.4 ± 26.5	90.2 ± 30.1	91.1 ± 30.9
$eGFR_{cysC}$,[m] mL/min/1.73m^2	82.2 ± 19.3	82.3 ± 19.0	78.8 ± 20.9
$eGFR_{cr-cysC}$,[m] mL/min/1.73m^2	79.4 ± 18.4	79.2 ± 18.1	76.0 ± 20.0

Data are reported as number of patients (%), mean ± SD or median (interquartile range).
ACE, angiotensin-converting enzyme; ATII, angiotensin IsI; cr, creatinine; CRP, C-reactive protein; cysC, cystatin C; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MET, metabolic equivalent task.
Two patients with BMI<18.5 kg/m^2 were not reported in this table.
[a] Defined as higher vocational education or university.
[b] Defined as ≥1 glass per week.
[c] Defined as three or more metabolic equivalent task (METs) during ≥5 days/week.
[d] Self-reported diagnosis by a physician, use of glucose-lowering drugs, or in case of elevated plasma glucose level (≥126 mmol/L in the case of patients who had fasted 4 hours or ≥200 mmol/L in the case of non-fasting patients).
[e] Blood pressure-lowering drugs: Anatomical Therapeutic Chemical Classification System (ATC) codes C02, C03, C07, C08, and C09.
[f] Glucose-lowering drugs: ATC code A10, A10A, A10B, A10X.
[g] Lipid-modifying drugs: ATC code C10, C10AA.
[h] Antithrombotic agents: ATC code B01.
[i] To convert the values for cholesterol to mg/dL, divide by 0.02586.
[j] To convert the values for triglycerides to mg/dL, divide by 0.01129.
[k] To convert the values for glucose to mg/dL, divide by 0.05551.
[l] To convert the values for creatinine to mg/dL, divide by 88.40.
[m] $eGFR_{cysC}$ and $eGFR_{cr-cysC}$ based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations from 2012.[13]

Baseline kidney function

At baseline, mean (SD) $eGFR_{cysC}$ was 83.3 (19.3) mL/min/1.73m^2 for men and 74.3 (18.8) mL/min/1.73m^2 for women. In obese compared to normal weight men and women the mean $eGFR_{cysC}$ was 81.1 versus 84.5 mL/min/1.73m^2 (P=0.006), and 69.1 versus 78.1 mL/min/1.73m^2 (P<0.001), respectively. Men with a high WC (≥102 cm) had a mean $eGFR_{cysC}$ of 81.8 mL/min/1.73m^2 compared to 84.9 mL/min/1.73m^2 in those with normal WC (<102 cm) (P<0.001). Women with high WC (≥88 cm) and normal WC (<88 cm) had mean $eGFR_{cysC}$ values of 73.6 and 76.8 mL/min/1.73m^2 (P=0.08).

Body mass index and kidney function decline

After 41 months of follow-up, mean (95% CI) decline in $eGFR_{cysC}$ was -4.61 (-5.06 to -4.17) mL/min/1.73m^2. Assuming a linear decline in kidney function, this corresponds to an annual decline of -1.34 mL/min/1.73m^2. Men and women had an annual $eGFR_{cysC}$ decline of -1.45 and -0.92 mL/min/1.73m^2, respectively (mean difference 0.53, 95% CI: 0.22 to 0.85). Annual rates of kidney function decline for normal weight, overweight and obese patients were-1.25, -1.30 and -1.59 mL/min/1.73m^2, respectively (Table 2). Rapid annual kidney function decline was observed in 25% of obese patients and 23% of normal weight patients (P=0.23). Obese versus normal weight men had an additional annual $eGFR_{cysC}$ decline of -0.42 (-0.85 to 0.02), corresponding to an additional 30% decline in kidney function. Obese versus normal weight women had an additional annual $eGFR_{cysC}$ decline -0.35 (-1.22 to 0.53) mL/min/1.73m^2, corresponding to an additional 45% decline in kidney function. Each 5 kg/m^2 increment of BMI was associated with an additional annual $eGFR_{cysC}$ decline of -0.35 mL/min/1.73m^2 in men and -0.21 mL/min/1.73m^2 in women, corresponding to 25% and 28% of the sex-specific mean annual kidney function decline in normal weight patients (Table 3). Supplementary Table S3 shows the adjusted analysis in more detail. Figure 1A depicts the continuous relation between BMI and annual kidney function decline for men and women. There was no effect measure modification between BMI and sex with regard to kidney function decline.

Table 2: Mean (95% CI) annual cystatin C based kidney function decline (mL/min/1.73m²) in 2408 post-myocardial infarction patients according to BMI and WC category, overall and for men and women separately.

	All patients	Normal weight[a] (ref)	Overweight	Obesity	Normal WC[b] (ref)	High WC
All patients	n=2408	n=527	n=1328	n=553	n=1022	n=1386
Crude	-1.34 (-1.46; -1.21)	-1.25 (-1.53; -0.98)	-1.33 (-1.50; -1.15)	-1.46 (-1.73; -1.19)	-1.19 (-1.38; -0.99)	-1.45 (-1.62; -1.29)
Model 1		-1.25 (-1.53; -0.98)	-1.29 (-1.47; -1.12)	-1.60 (-1.87; -1.33)	-1.19 (-1.38; -0.99)	-1.57 (-1.75; -1.39)
Model 2		-1.25 (-1.53; -0.98)	-1.30 (-1.48; -1.13)	-1.59 (-1.87; -1.32)	-1.19 (-1.38; -0.99)	-1.57 (-1.74; -1.39)
Men	n=1914	n=419	n=1116	n=379	n=914	n=1000
Crude	-1.45 (-1.59; -1.31)	-1.38 (-1.68; -1.09)	-1.39 (-1.58; -1.21)	-1.69 (-2.00; -1.38)	-1.25 (-1.46; -1.05)	-1.63 (-1.82; -1.44)
Model 1		-1.38 (-1.69; -1.09)	-1.41 (-1.59; -1.22)	-1.82 (-2.14; -1.51)	-1.25 (-1.46; -1.05)	-1.65 (-1.84; -1.45)
Model 2		-1.38 (-1.69; -1.09)	-1.41 (-1.59; -1.23)	-1.80 (-2.12; -1.49)	-1.25 (-1.46; -1.05)	-1.63 (-1.82; -1.44)
Women	n=494	n=108	n=212	n=174	n=108	n=386
Crude	-0.92 (-1.20; -0.63)	-0.75 (-1.42; -0.08)	-0.97 (-1.45; -0.49)	-0.96 (-1.49; -0.43)	-0.61 (-1.28; 0.06)	-1.00 (-1.36; -0.65)
Model 1		-0.75 (-1.42; -0.08)	-0.92 (-1.41; -0.44)	-0.94 (-1.47; -0.40)	-0.61 (-1.28; 0.06)	-0.95 (-1.32; -0.59)
Model 2		-0.75 (-1.42; -0.08)	-1.03 (-1.54; -0.53)	-1.09 (-1.66; -0.52)	-0.61 (-1.28; 0.06)	-1.01 (-1.38; -0.64)

BMI, body-mass index; CI, confidence interval; WC, waist circumference.
Normal weight BMI 18.5 - 24.9, overweight BMI 25.0 - 29.9, obesity BMI ≥30.0 kg/m². Normal and high WC <88 and ≥88 cm for women and <102 and ≥102 cm for men. Kidney function based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation from 2012.[23]
Two patients with BMI<18.5 kg/m² were not reported in this table. Adjusted variables were fixed at the mean value of the reference group, hence the results of the reference category are equal across models.
[a] Reference: annual kidney function decline in normal weight patients.
[b] Reference: annual kidney function decline in normal WC patients.
Model 1: adjusted for treatment group, age and sex.
Model 2: model 1 plus additional adjustment for current smoking, alcohol use, level of education.

Table 3: Association of BMI and WC with annual cystatin C based kidney function decline in 2410 post-myocardial infarction patients, overall and for men and women separately.

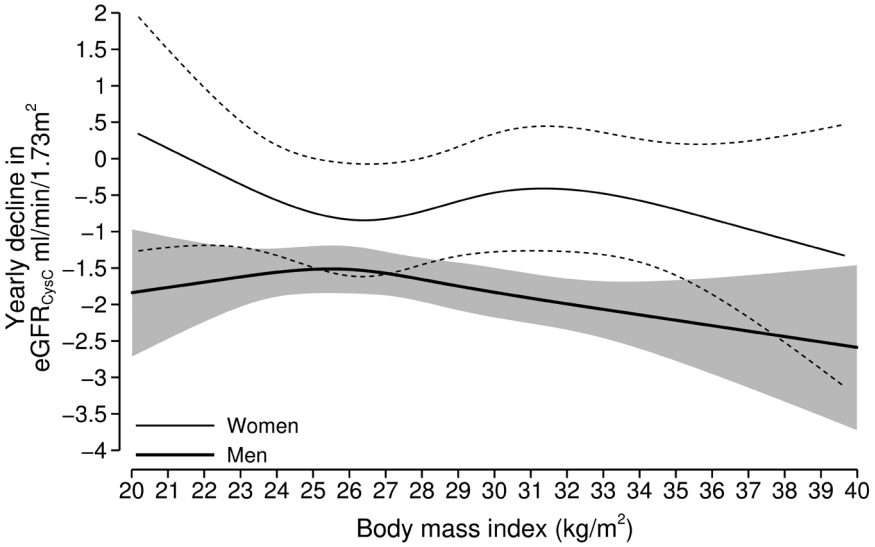
	Additional annual $eGFR_{cysC}$ decline, mean (95%-CI)		
	Total, n=2410	**Men, n=1914**	**Women, n=496**
Per 5 kg/m² increment of BMI			
Crude	-0.20 (-0.37; -0.02)	-0.27 (-0.48; -0.06)	-0.15 (-0.48; 0.19)
Model 1	-0.28 (-0.46; -0.11)	-0.36 (-0.57; -0.15)	-0.15 (-0.49; 0.19)
Model 2	-0.28 (-0.46; -0.10)	-0.35 (-0.56; -0.14)	-0.21 (-0.55; 0.14)
Per 10 cm increment of WC			
Crude	-0.24 (-0.36; -0.11)	-0.19 (-0.35; -0.04)	-0.19 (-0.46; 0.08)
Model 1	-0.21 (-0.34; -0.08)	-0.21 (-0.37; -0.06)	-0.19 (-0.46; 0.08)
Model 2	-0.20 (-0.34; -0.07)	-0.21 (-0.36; -0.06)	-0.22 (-0.49; 0.06)

BMI, body-mass index; CI, confidence interval; $eGFR_{cysC}$, Cystatin C based estimated glomerular filtration rate; WC, waist circumference.
Kidney function based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, 2012.[13]
Model 1: adjusted for treatment group, age and sex.
Model 2: model 1, additionally adjusted for current smoking, alcohol use, level of education

Waist circumference and kidney function decline

Men and women with high versus normal WC had a faster annual decline in kidney function (Table 2). In men, the additional decline in eGFR (95% CI) was -0.39 (-0.66 to -0.13) mL/min/1.73m²; for women it was -0.40 (-1.17 to 0.36) mL/min/1.73m². Among patients with high and normal WC, 26% and 21% showed rapid kidney function decline, respectively (P=0.003). In regression analysis, a squared WC term was significant in men (P=0.03) but not in women (P=0.2). For each 10 cm increment of WC there was an additional annual kidney function decline of -0.21 mL/min/1.73m² in men and -0.22 mL/min/1.73m² in women (Table 3 and Supplementary Table S3). Figure 1B depicts the continuous relation between WC and annual kidney function decline for men and women.

A.



B.

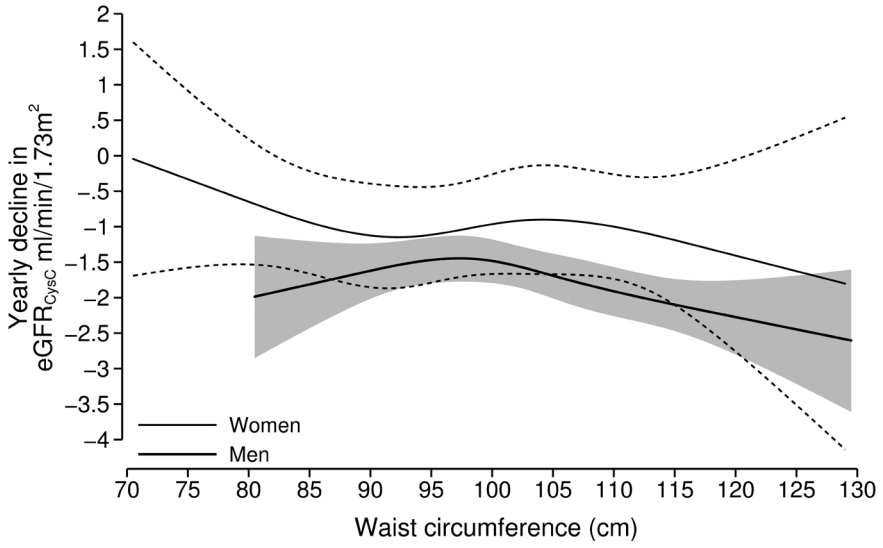


Figure 1. A. Association between body mass index (BMI) and B. waist circumference (WC) and annual kidney function decline for men and women. Linear regression coefficients for annual kidney function decline according to BMI or WC were modelled by separate restricted cubic splines. Patients with extreme values of BMI [<20 kg/m^2 (N=22, 0.9%) and >40 kg/m^2 (N=11, 0.5%)], or WC [<70 for women, <80 for men with BMI <20 kg/m^2 (n=2 and n=1) and >130 cm (n=18)] were excluded. The model was adjusted for age, treatment group and current smoking.

Sensitivity analyses

In addition to model 2, further adjustment for diabetes attenuated the association of BMI (and WC) with kidney function decline. The regression coefficient per 5 kg/m^2 BMI changed from -0.28 to -0.20 (Supplementary Table S4). Additional adjustment for systolic blood pressure or LDL-cholesterol did not change the association. Adjustment for use of RAS blocking drugs or physical activity did not essentially change the results. There was no evidence for effect modification between BMI or WC and treatment group with regard to kidney function decline (data not shown). When WC, instead of BMI, was taken as determinant, results were comparable. On average, BMI and WC did not change during follow-up, with a mean (SD) change of 0.03 (1.67) kg/m^2 and 0.14 (5.99) cm. Change in BMI was not associated with annual $eGFR_{cysC}$ decline. The regression coefficient for each unit decline in BMI was -0.052 (-0.129 to 0.024). Likewise, decline in WC was not associated with $eGFR_{cysC}$ decline. Finally, taking $eGFR_{cr-cysC}$ as outcome, resulted in slightly weaker effect estimates (Supplementary Table S5 and S6).

DISCUSSION

This is the first study to show a progressive association between adiposity and kidney function decline in stable post-MI patients receiving optimal pharmacological treatment. The mean annual decline in kidney function was -1.45 mL/min/1.73m^2 for men and -0.92 mL/min/1.73m^2 for women. Obese men and women showed, on average, 30% and 45% faster annual kidney function decline than individuals of normal weight. Each 5 kg/m^2 increment of BMI was associated with an additional annual kidney function decline of -0.35 mL/min/1.73m^2 in men and -0.21 mL/min/1.73m^2 in women. Finally, men and women with high versus normal WC experienced a more rapid decline in kidney function.

The annual kidney function decline of -1.3 mL/min/1.73m^2 observed in our study is lower than the -2.2 mL/min/1.73m^2 for post-MI patients found in the Prevention of Renal and Vascular End-stage Disease study, possibly because the patients in our cohort received more optimal cardiovascular drug treatment.[5] Other researchers have reported a mean annual eGFR decline of-1.0 mL/min/1.73m^2 in a community-based cohort (mean age 55 years) and -1.8 mL/min/1.73m^2 in healthy individuals (mean age 72 years).[19, 20] The size of the association between high BMI and WC on kidney function decline that we found was small. However, a persistently slower kidney function decline may postpone or prevent CKD in patients at high risk, which is clinically relevant.

In addition, we recently showed in the Alpha Omega cohort a linear increase in mortality risk (cardiovascular and non-cardiovascular) for patients with an eGFR below 80 mL/min/1.73m^2.[4] Preservation of kidney function is therefore important, especially in these high-risk patients.

Few studies have examined the association between BMI and kidney function decline. One study found that in younger healthy adults, being overweight or obese was associated with 1.50 and 1.85 times higher risk of rapid kidney function decline (>3% eGFR per year) compared to normal weight individuals.[2] Others have shown that being overweight at a younger age (26 years), compared to older age (60 years), is associated with double the risk of progression to CKD stage 3–5 by the age of 65.[21] Interestingly, weight loss in obese patients improves kidney function. In morbidly obese patients aged between 18 and 60 years old with glomerular hyperfiltration, kidney function normalized after weight loss by gastric bypass surgery.[22] We found no association between change in BMI and kidney function decline. However, BMI hardly changed during the relative short follow-up and we had no information whether weight loss was intentional or not. In our study, men had a faster rate of kidney function decline compared to women at each BMI level, but we found no effect modification. In contrast, one meta-analysis found that obese women versus men had a higher risk of CKD compared to normal-weight individuals.[23]

In addition to BMI, we evaluated the effect of WC, since it is a more accurate measure for visceral fat.[1] The correlation coefficient of 0.8 between BMI and WC observed here was similar to that seen in a study which assessed patients with metabolic syndrome (mean age 68 years).[24,25] In line with our results, others reported that individuals with high versus low WC had a 24% versus 20% risk of annual eGFR decline of >5%, in a multi-ethnic non-diabetic population.[25] We found for men an indication of an inverse U-shaped association between WC (or BMI) and $eGFR_{cysC}$ decline. A possible explanation is that low weight can be a proxy of underlying disease, which is particularly relevant in elderly patients. However, the wide 95% confidence intervals reflect the great uncertainty for the lower ranges of WC and BMI.

In contrast to our results, some studies have shown that overweight or mild obesity is reno-protective compared to normal weight, both in patients with eGFR <60 or ≥60 mL/min/1.73m^2.[9,10] In contrast to our study, this cohort consists of US army veterans (95% men, mean age 73y), with a lower mean eGFR of 48 mL/min/1.73m^2, and a large prevalence of malignancies and lung disease. Moreover, these studies did not control for smoking, which may have contributed to an underestimation of the effect of obesity, while smokers in general have lower BMI.[26]

Various mechanisms have been proposed through which overweight and obesity could promote accelerated loss of kidney function, in addition to diabetic and hypertensive nephropathy. Obesity is associated with a state of low-grade systemic inflammation, and has been shown to cause kidney damage and eventually fibrosis via the activity of pro-inflammatory cytokines such as transforming growth factor β.[27]

This study has limitations. First, the study design is observational, and therefore no causal inferences can be made. Second, we estimated kidney function at only two time points, which reduces precision of the estimates. Third, we did not measure kidney function directly. However, direct measurement of GFR is cumbersome, expensive, and rarely available in large epidemiological studies, and several reports have suggested that even iothalamate measurement can have daily variations of up to 8%.[28] Fourth, no information was available on proteinuria, an important predictor of kidney function decline. Finally, our results are applicable to post-MI patients and may therefore not be generalizable to other populations. However, both the prevalence of obesity and the prevalence of cardiovascular disease show an increasing trend worldwide, and our cohort of patients therefore represents a growing patient group.

The study has several strengths. First, to our knowledge this is the only large study that explored the association of both BMI and WC with kidney function decline in post-MI-patients receiving optimal pharmacological drug treatment, and for men and women separately. Second[4], we measured cysC, which is currently the most accurate marker to estimate GFR, and in contrast to creatinine based eGFR is most likely not affected by glomerular hyperfiltration.[11, 29]

In conclusion, we found in older stable post-MI patients that high BMI and WC were associated with progressive cysC-based kidney function decline, despite cardiovascular drug treatment with antihypertensive, cholesterol-lowering, antithrombotic and glucose-lowering drugs. Further research is needed to study whether prevention of obesity or weight loss intervention on-top of cardiovascular drug treatment can slow down the accelerated kidney function decline in post-MI patients.

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DISCLOSURES

EH is a member of the Guideline Committee of the Dutch Federation of Nephrology. JG received research funding from Unilever R&D for epidemiological studies of dietary fatty acids and is a member of the Standing Committee on Nutrition of the Dutch Health Council, Working Group on Minerals of the European Food and Safety Authority, and Dutch Academy for Nutritional Sciences, and is a Fellow of the American Heart Association. DK received research funding from the Royal Netherlands Academy of Arts and Sciences and is Member of the Dutch Academy of Nutritional Sciences. KE, TS, JF, FD and EG report that they have no disclosures.

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AUTHOR CONTRIBUTIONS

KE, JG, EG, DK and EH contributed to conception and design of the manuscript. KE, JG, EG, DK and EH contributed to acquisition, analysis and interpretation, and drafted the manuscript. TS, FD and JF contributed to interpretation. All authors critically revised the manuscript, all gave final approval and all agreed to be accountable for all aspects of work, ensuring integrity and accuracy.

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SUPPLEMENTARY DATA

Table S1: Baseline characteristics of 2410 post-myocardial infarction patients, stratified by normal and high waist circumference, according to the sex-specific WHO classification.

Waist circumference	Normal (n=1024)	High (n=1386)
Age, years	68.8 ± 5.3	68.9 ± 5.5
Men, no.(%)	914 (89.3)	1000 (72.2)
Ethnicity, white, no. (%)	1010 (98.6)	1372 (99.0)
Higher education,[a] no. (%)	153 (14.9)	144 (10.4)
Current smoking, no. (%)	169 (16.5)	215 (15.5)
Alcohol use,[b] n (%)	791 (77.2)	953 (68.8)
Height, cm	172.3 ± 7.5	172.2 ± 8.6
Weight, kg	74.8 ± 8.9	87.6 ± 11.9
Body mass index, kg/m^2	25.2 ± 2.2	29.5 ± 3.4
Waist circumference, cm	93.7 ± 6.5	106.8 ± 8.4
Physically active,[c] n (%)	256 (25.0)	274 (19.8)
Time since myocardial infarction, yr	3.9 (1.8–6.3)	4.1 (2.1–6.6)
Diabetes,[d] n (%)	133 (13.0)	311 (22.4)
Systolic blood pressure, mmHg	142.8 ± 21.4	143.6 ± 21.3
Diastolic blood pressure, mmHg	80.6 ± 10.6	82.0 ± 10.7
Antihypertensive drugs,[e] n (%)	851 (83.1)	1246 (89.9)
ACE inhibitors/ATII blockers	499 (48.7)	801 (57.8)
Beta blockers	640 (62.5)	935 (67.5)
Calcium channel blockers	168 (16.4)	294 (21.2)
Diuretics	135 (13.2)	364 (26.3)
Glucose-lowering drugs,[f] n (%)	101 (9.9)	214 (15.4)
Insulin analogues	22 (2.1)	82 (5.9)
Oral glucose-lowering drugs	85 (8.3)	166 (12.0)
Lipid-modifying drugs,[g] n (%)	873 (85.3)	1203 (86.8)
Statins	866 (84.6)	1194 (86.1)
Antithrombotic agents,[h] n (%)	999 (97.6)	1354 (97.7)

Table S1: Continued

Waist circumference	Normal (n=1024)	High (n=1386)
Total cholesterol,[i] mmol/L	4.76 ± 0.90	4.90 ± 0.95
HDL,[i] mmol/L	1.29 ± 0.33	1.23 ± 0.32
LDL,[i] mmol/L	2.72 ± 0.78	2.76 ± 0.81
Triglycerides,[j] mmol/L	1.46 (1.09–2.00)	1.77 (1.33–2.48)
Plasma glucose,[k] mmol/L	5.7 ± 1.7	6.2 ± 2.1
High-sensitivity CRP, mg/L	1.26 (0.67–2.55)	2.15 (0.97–4.24)
Serum cystatin C, mg/L	0.95 ± 0.23	0.99 ± 0.25
Serum creatinine,[l] µmol/L	89.5 ± 27.3	90.5 ± 30.8
$eGFR_{cysC}$,[m] mL/min/1.73m^2	84.0 ± 18.9	79.6 ± 19.8
$eGFR_{cr-cysC}$,[m] mL/min/1.73m^2	80.9 ± 18.0	76.7 ± 19.0

Data are reported as number of patients (%), mean ± SD or median (interquartile range).
ACE, angiotensin-converting enzyme; ATII, angiotensin II; cr, creatinine; CRP, C-reactive protein; cysC, cystatin C; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MET, metabolic equivalent task.
[a] Defined as higher vocational education or university.
[b] Defined as ≥1 glass per week.
[c] Defined as three or more metabolic equivalent task (METs) during ≥5 days/week.
[d] Self-reported diagnosis by a physician, use of glucose-lowering drugs, or in case of elevated plasma glucose level (≥126 mmol/L in the case of patients who had fasted 4 hours or ≥200 mmol/L in the case of non-fasting patients).
[e] Blood pressure-lowering drugs: Anatomical Therapeutic Chemical Classification System (ATC) codes C02, C03, C07, C08, and C09.
[f] Glucose-lowering drugs: ATC code A10, A10A, A10B, A10X.
[g] Lipid-modifying drugs: ATC code C10, C10AA.
[h] Antithrombotic agents: ATC code B01.
[i] To convert the values for cholesterol to mg/dL, divide by 0.02586.
[j] To convert the values for triglycerides to mg/dL, divide by 0.01129.
[k] To convert the values for glucose to mg/dL, divide by 0.05551.
[l] To convert the values for creatinine to mg/dL, divide by 88.40.
[m] $eGFR_{cysC}$ and $eGFR_{cr-cysC}$ based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations from 2012.[13]

Table S2: Baseline characteristics of all 2410 post-myocardial infarction patients, overall and for men and women separately.

	All patients (n=2410)	Men (n=1914)	Women (n=496)
Age, years	68.9 ± 5.4	68.5 ± 5.3	70.2 ± 5.6
Ethnicity, white, no. (%)	2382 (98.8)	1894 (99.0)	488 (98.4)
Higher education,[a] no. (%)	297 (12.3)	272 (14.2)	25 (5.0)
Current smoking, no. (%)	384 (15.9)	308 (16.1)	76 (15.3)
Alcohol use,[b] n (%)	1744 (72.4)	1497 (78.2)	247 (49.8)
Height, cm	172.2 ± 8.1	174.8 ± 6.4	162.3 ± 6.2
Weight, kg	82.2 ± 12.4	84.1 ± 11.6	74.8 ± 12.6
Body mass index, kg/m^2	27.7 ± 3.6	27.5 ± 3.3	28.4 ± 4.6
Waist circumference, cm	101.2 ± 10.0	102.5 ± 9.1	96.4 ± 11.6
Physically active,[c] n (%)	530 (22.0)	442 (23.1)	88 (17.7)
Time since myocardial infarction, yr	4.0 (2.0–6.4)	4.1 (2.1–6.6)	3.5 (1.7–6.1)
Diabetes,[d] n (%)	444 (18.4)	330 (17.2)	114 (23.0)
Systolic blood pressure, mmHg	143.3 ± 21.4	143.5 ± 20.9	142.3 ± 23.0
Diastolic blood pressure, mmHg	81.4 ± 10.7	81.8 ± 10.6	79.8 ± 11.1
Antihypertensive drugs,[e] n (%)	2097 (87.0)	1644 (85.9)	453 (91.3)
ACE inhibitors/ATII blockers	1300 (53.9)	1013 (52.9)	287 (57.9)
Beta blockers	1575 (65.4)	1230 (64.3)	345 (69.6)
Calcium channel blockers	462 (19.2)	362 (18.9)	100 (20.2)
Diuretics	499 (20.7)	333 (17.4)	166 (33.5)
Glucose-lowering drugs,[f] n (%)	315 (13.1)	227 (11.9)	88 (17.7)
Insulin analogues	104 (4.3)	69 (3.6)	35 (7.1)
Oral glucose-lowering drugs	251 (10.4)	186 (9.7)	65 (13.1)
Lipid-modifying drugs,[g] n (%)	2076 (86.1)	1648 (86.1)	428 (86.3)
Statins	2060 (85.5)	1633 (85.3)	427 (86.1)
Antithrombotic agents,[h] n (%)	2353 (97.6)	1875 (98.0)	478 (96.4)
Total cholesterol,[i] mmol/L	4.84 ± 0.93	4.75 ± 0.90	5.18 ± 0.98
HDL,[i] mmol/L	1.26 ± 0.33	1.22 ± 0.30	1.41 ± 0.37
LDL,[i] mmol/L	2.74 ± 0.80	2.71 ± 0.78	2.88 ± 0.84
Triglycerides,[j] mmol/L	1.63 (1.22–2.28)	1.62 (1.20–2.25)	1.65 (1.27–2.39)
Plasma glucose,[k] mmol/L	6.0 ± 2.0	6.0 ± 1.9	6.1 ± 2.2

Table S2: Continued

	All patients (n=2410)	Men (n=1914)	Women (n=496)
High-sensitivity CRP, mg/L	1.66 (0.82–3.61)	1.52 (0.80–3.33)	2.28 (0.93–4.48)
Serum cystatin C, mg/L	0.97 ± 0.24	0.96 ± 0.24	1.00 ± 0.27
Serum creatinine,[l] µmol/L	90.1 ± 29.3	92.6 ± 29.7	79.8 ±25.2
$eGFR_{cysC}$,[m] mL/min/1.73m^2	81.5 ± 19.6	83.3 ± 19.3	74.3 ± 18.8
$eGFR_{cr-cysC}$,[m] mL/min/1.73m^2	78.5 ±18.7	80.3 ± 18.4	71.0 ± 17.8

Data are reported as number of patients (%), mean ± SD or median (interquartile range).
ACE, angiotensin-converting enzyme; ATII, angiotensin II; cr, creatinine; CRP, C-reactive protein; cysC, cystatin C; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MET, metabolic equivalent task.
[a] Defined as higher vocational education or university.
[b] Defined as ≥1 glass per week.
[c] Defined as three or more metabolic equivalent task (METs) during ≥5 days/week.
[d] Self-reported diagnosis by a physician, use of glucose-lowering drugs, or in case of elevated plasma glucose level (≥126 mmol/L in the case of patients who had fasted 4 hours or ≥200 mmol/L in the case of non-fasting patients).
[e] Blood pressure-lowering drugs: Anatomical Therapeutic Chemical Classification System (ATC) codes C02, C03, C07, C08, and C09.
[f] Glucose-lowering drugs: ATC code A10, A10A, A10B, A10X.
[g] Lipid-modifying drugs: ATC code C10, C10AA.
[h] Antithrombotic agents: ATC code B01.
[i] To convert the values for cholesterol to mg/dL, divide by 0.02586.
[j] To convert the values for triglycerides to mg/dL, divide by 0.01129.
[k] To convert the values for glucose to mg/dL, divide by 0.05551.
[l] To convert the values for creatinine to mg/dL, divide by 88.40.
[m] $eGFR_{cysC}$ and $eGFR_{cr-cysC}$ based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations from 2012.[13]

Table S3: Association of BMI and WC with annual cystatin C based kidney function decline in 2410 post-MI patients. Analyses are adjusted one by one for confounding factors.

Model	BMI, per 5 kg/m²	WC, per 10 cm
Crude	-0.20 (-0.37 to -0.02)	-0.24 (-0.36 to -0.11)
Treatment group	-0.20 (-0.37 to -0.02)	-0.23 (-0.36 to -0.11)
Model 1	-0.27 (-0.45 to -0.09)	-0.21 (-0.34 to -0.08)
Model 1 + smoking	-0.29 (-0.46 to -0.11)	-0.21 (-0.34 to -0.08)
Model 1 + alcohol use	-0.28 (-0.46 to -0.10)	-0.21 (-0.34 to -0.07)
Model 1 + education	-0.28 (-0.45 to -0.10)	-0.20 (-0.33 to -0.07)
Model 2 (full model)	-0.28 (-0.46 to -0.10)	-0.20 (-0.34 to -0.06)

BMI, body-mass index; WC, waist circumference.
Model 1: adjusted for treatment group, age and sex.
Model 2: model 1 plus additional adjustment for current smoking, alcohol use, and level of education.

Table S4: Association of BMI and WC with annual cystatin C based kidney function decline in 2410 post-MI patients. Analyses are adjusted for factors in the causal path (diabetes, systolic blood pressure, LDL-cholesterol).

Model	BMI, per 5 kg/m²	WC, per 10 cm
Crude	-0.20 (-0.37 to -0.02)	-0.24 (-0.36 to -0.11)
Model 1	-0.27 (-0.45 to -0.09)	-0.21 (-0.34 to -0.08)
Model 2	-0.28 (-0.46 to -0.10)	-0.20 (-0.34 to -0.06)
Model 2 + diabetes	-0.20 (-0.38 to -0.02)	-0.14 (-0.28 to -0.01)
Model 2 + systolic blood pressure	-0.27 (-0.45 to -0.09)	-0.20 (-0.33 to -0.07)
Model 2 + LDL	-0.28 (-0.47 to -0.10)	-0.20 (-0.34 to -0.06)

BMI, body-mass index; LDL, low-density lipoprotein; WC, waist circumference
Model 1: adjusted for treatment group, age and sex.
Model 2: model 1 plus additional adjustment for current smoking, alcohol use, and level of education.

Table S5: Mean (95% CI) annual creatinine-cystatin C based kidney function decline (mL/min/1.73m²) in 2328 post-myocardial infarction patients according to BMI or WC category, overall and for men and women separately.

	All patients	Normal weight (ref)	Overweight[a]	Obesity[a]	Normal WC (ref)	High WC[b]
All patients	n=2328	n=511	n=1288	n=529	n=996	n=1332
Crude	-1.73 (-1.89 to -1.58)	-1.71 (-2.04 to -1.39)	-1.67 (-1.88 to -1.47)	-1.92 (-2.24 to -1.60)	-1.63 (-1.86 to -1.39)	-1.82 (-2.02 to -1.62)
Model 1		-1.71 (-2.04 to -1.39)	-1.66 (-1.86 to -1.45)	-1.94 (-2.27 to-1.62)	-1.63 (-1.86 to -1.39)	-1.79 (-2.01 to -1.58)
Model 2		-1.71 (-2.04 to -1.39)	-1.66 (-1.86 to -1.45)	-1.93 (-2.26 to -1.60)	-1.63 (-1.86 to -1.39)	-1.79 (-2.00 to -1.57)
Men	n=1877	n=416	n=1092	n=369	n=902	n=975
Crude	-1.70 (-1.86 to -1.54)	-1.73 (-2.07 to -1.38)	-1.66 (-1.87 to -1.45)	-1.80 (-2.16 to -1.44)	-1.62 (-1.85 to -1.39)	-1.78 (-2.00 to -1.56)
Model 1		-1.73 (-2.07 to -1.38)	-1.66 (-1.87 to -1.45)	-1.88 (-2.25 to -1.51)	-1.62 (-1.85 to -1.39)	-1.79 (-2.01 to -1.56)
Model 2		-1.73 (-2.07 to -1.38)	-1.66 (-1.87 to -1.45)	-1.86 (-2.23 to -1.49)	-1.62 (-1.85 to -1.39)	-1.77 (-1.99 to -1.54)
Women	n=451	n=95	n=196	n=160	n=94	n=357
Crude	-1.86 (-2.27 to -1.45)	-1.65 (-2.55 to -0.76)	-1.72 (-2.35 to -1.10)	-2.18 (-2.87 to -1.49)	-1.68 (-2.58 to -0.78)	-1.92 (-2.38 to -1.46)
Model 1		-1.65 (-2.55 to -0.76)	-1.63 (-2.27 to -0.99)	-2.16 (-2.84 to -1.47)	-1.68 (-2.58 to -0.78)	-1.84 (-2.31 to -1.36)
Model 2		-1.65 (-2.55 to -0.76)	-1.63 (-2.29 to -0.98)	-2.31 (-3.05 to -1.56)	-1.68 (-2.58 to -0.78)	-1.88 (-2.37 to -1.39)

BMI, body-mass index; CI, confidence interval; WC, waist circumference
Normal weight BMI 18.5 - 24.9, overweight BMI 25.0 - 29.9, obesity BMI ≥30.0 kg/m². Normal and high WC <88 and ≥88 cm for women and <102 and ≥102 cm for men. Kidney function based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) combined creatinine-cystatin C equation from 2012.[13]
[a] Reference: annual kidney function decline in normal weight patients. Adjusted variables were fixed at the mean value of the reference group, hence the results of the reference category are equal across models.
[b] Reference: annual kidney function decline in normal WC patients.
Model 1: adjusted for treatment group, age and sex (if not stratified for).
Model 2: model 1 plus additional adjustment for current smoking, alcohol use, level of education.

Table S6: Association of BMI and WC with annual creatinine-cystatin C based kidney function decline in 2328 post-myocardial infarction patients, overall and for men and women separately.

	Additional annual $eGFR_{cr-cysC}$ decline, mean (95%-CI)		
	Total, n=2328	**Men, n=1877**	**Women, n=451**
Per 5 kg/m² increment of BMI			
Crude	-0.17 (-0.38 to 0.04)	-0.17 (-0.41 to 0.07)	-0.16 (-0.60 to 0.28)
Model 1	-0.19 (-0.41 to 0.02)	-0.22 (-0.47 to 0.02)	-0.16 (-0.60 to 0.28)
Model 2	-0.19 (-0.40 to 0.02)	-0.21 (-0.46 to 0.03)	-0.22 (-0.67 to 0.23)
Per 10 cm increment of WC			
Crude	-0.12 (-0.27 to 0.04)	-0.12 (-0.29 to 0.06)	-0.19 (-0.55 to 0.16)
Model 1	-0.14 (-0.30 to 0.01)	-0.13 (-0.30 to 0.05)	-0.18 (-0.53 to 0.17)
Model 2	-0.14 (-0.30 to 0.02)	-0.12 (-0.30 to 0.05)	-0.21 (-0.57 to 0.14)

BMI, body-mass index; CI, confidence interval; $eGFR_{cr-cysC}$, combined creatinine-cystatin C based estimated glomerular filtration rate; WC, waist circumference.
Kidney function based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) combined creatinine-cystatin C equation, 2012.[13]
Model 1: adjusted for four randomized treatment groups, age and sex (if not stratified for).
Model 2: model 1, additionally adjusted for smoking, alcohol use, level of education.

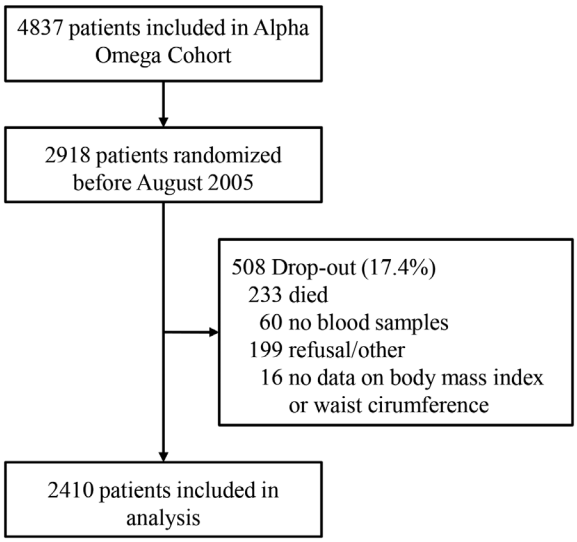

Figure S1: Flow chart of 2410 patients included in the present study. The patients randomized before August 2005 are considered a random sample of the total population of 4837 patients.

Chapter 4 –

Dietary protein intake and kidney function decline after myocardial infarction: the Alpha Omega Cohort

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ABSTRACT

Background: Post-myocardial infarction (MI) patients have a doubled rate of kidney function decline compared to the general population. We investigated the extent to which high intake of total, animal, and plant protein are risk factors for accelerated kidney function decline in older stable post-MI patients.

Methods: We analyzed 2255 post-MI patients (age 60-80y, 80% men) of the Alpha Omega Cohort. Dietary data were collected with a biomarker-validated 203-item food frequency questionnaire. At baseline and 41 months, we estimated glomerular filtration rate based on the CKD-EPI equations for serum cystatin C ($eGFR_{cysC}$) alone and both creatinine and cystatin C ($eGFR_{cr-cysC}$).

Results: Mean (SD) baseline $eGFR_{cysC}$ and $eGFR_{cr-cysC}$ were 82 (20) and 79 (19) mL/min/1.73m^2. Of all patients, 16% were current smokers, and 19% had diabetes. Mean (SD) total protein intake was 71 (19) g/day, of which 2/3 was animal and 1/3 plant protein. After multivariable adjustment, including age, sex, total energy, smoking, diabetes, systolic blood pressure, renin-angiotensin system blocking drugs, and fat, each incremental total daily protein intake of 0.1 g/kg ideal body weight was associated with an additional annual $eGFR_{cysC}$ decline of -0.12 (95%-CI: -0.19; -0.04) mL/min/1.73m^2, and was similar for animal and plant protein. Patients with a daily total protein intake of ≥1.20 compared to <0.80 g/kg ideal body weight had a 2-fold faster annual $eGFR_{cysC}$ decline of -1.60 versus -0.84 mL/min/1.73m^2. Taking $eGFR_{cr-cysC}$ as outcome showed similar results. Strong linear associations were confirmed by restricted cubic spline analyses.

Conclusion: A higher protein intake was significantly associated with a more rapid kidney function decline in post-MI patients.

INTRODUCTION

In the European population ≥45 years, the prevalence of chronic kidney disease (CKD), defined as estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m^2, is high at 11%.[1] CKD is an independent risk factor for cardiovascular morbidity and mortality.[2, 3] Post-myocardial infarction (MI) patients, compared to the general population, have a doubled rate of annual kidney function decline of about 2.0 mL/min/1.73m^2, and are thus at risk for CKD.[4] Classic cardiovascular risk factors, such as diabetes, smoking and hypertension can only explain part of the accelerated kidney function decline. Identification of novel modifiable risk factors is important for targeted prevention of kidney function decline and may improve life expectancy in post-MI patients.

Experimental animal studies showed that long-term high levels of protein may cause glomerular hyperfiltration and pro-inflammatory gene expression, both well known risk factors for CKD progression.[5, 6] In humans, several studies showed that a high protein diet may exacerbate proteinuria, an independent risk factor of accelerated kidney function decline, although this was not confirmed by others.[7-9] Consequently, current Kidney Disease Improving Global Outcomes (KDIGO) guidelines recommend to limit daily total protein intake to <1.30 g/kg body weight in adults at risk for CKD, and advise to restrict protein intake to 0.60-0.80 g/kg/day in patients with diabetes or eGFR <30 mL/min/1.73m^2.[10, 11] The Modification of Diet in Renal Disease intervention study suggested that dietary protein restriction may slow down kidney function decline in patients with an eGFR between 25 and 55 mL/min/1.73m^2.[12]

From a preventive perspective it is of interest to know whether protein restriction in patients with normal or mildly impaired kidney function retards kidney function decline. Moreover, recommendations are lacking regarding relative animal or plant protein restriction.

The aim of the present study was to determine whether total protein, and its components animal and plant protein, are risk factors for accelerated kidney function decline in stable older post-MI patients with normal or mildly impaired kidney function.

MATERIALS AND METHODS

Participants

The Alpha Omega Cohort is a prospective study of 4837 Dutch patients aged 60-80 years with a clinically diagnosed myocardial infarction (MI) up to 10 years before study entry, on standard cardiovascular drug treatment according

to the latest international guidelines.[13,14] Major exclusion criteria were severe heart failure, unintended weight loss of ≥5 kg the previous year, and diagnosis of cancer with a life expectancy <1 year. During the first 41 months of follow-up, patients took part in an experimental study of low-dose omega-3 fatty acids (Alpha Omega Trial), as described elsewhere.[15] For the present study, we included patients with available blood samples at baseline and after 41 months of follow-up. Owing to financial constraints, a second blood sample was taken only of patients who were enrolled in the trial up to August 2005 (n=2918). From these 2918 patients we excluded those who died during follow-up (n=233), and who had missing blood samples or refused further participation (n=259). In addition, patients were excluded with missing dietary data (n=171) or implausible high or low energy intake (<800 or >8000 kcal/day for men, <600 or >6000 kcal/day for women; n=7), yielding 2248 patients for the present analysis (Supplementary Figure S1). The Alpha Omega Cohort study was registered at ClinicalTrials.gov no. NCT03192410. This study was conducted in accordance with the Helsinki Declaration and was approved by a central Medical Ethics Committee in the Netherlands. Written informed consent was obtained from all patients. Reporting of this study was performed in accordance with the STROBE guidelines for cohort studies.[16]

Data collection

Patients were interviewed and physically examined by trained research nurses at baseline and after 41 months. Information on demographic variables, lifestyle habits, and medical history was collected by self-administered questionnaires as previously described.[17] High blood pressure was defined according to the latest European Society of Cardiology guideline: a systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg.[18] Diabetes mellitus was considered present in case of a self-reported physician diagnosis, use of glucose-lowering drugs, and/or hyperglycemia (serum glucose ≥7.0 mmol/L for patients who had fasted ≥4 hours or ≥11.1 mmol/L for non-fasting patients). Body-mass index (BMI) was calculated as weight (kg) divided by the squared height (m) and obesity was defined as BMI ≥30 kg/m^2.[19] Physical activity was assessed by the Physical Activity Scale for the Elderly (PASE), a validated self-reported questionnaire for persons ≥65 years.[20] Medication was coded according to the Anatomical Therapeutic Chemical Classification (ATC) System. Standardized blood handling procedures, and determination of lipid and glucose levels were described in detail elsewhere.[17]

Dietary data

We collected dietary data using a 203-item food frequency questionnaire (FFQ), specifically developed for the Alpha Omega Trial.[15] The FFQ is an extended and adapted version of a reproducible and biomarker-validated FFQ.[21,22] Patients reported

their habitual food intake during the previous month, including information on frequency, amount, type and preparation methods of food. Questionnaires were checked by trained dieticians and patients were contacted by telephone in case of missing or unclear information. The 2006 Dutch food-composition database was used to convert food consumption into intake of energy, protein and other nutrients.[23] Dietary protein intake was collected at baseline, and we did not consider changes of intake during follow-up. Previous studies showed that the dietary pattern remained stable, especially at older age, over a timespan up to seven years.[24] We divided total protein intake into animal and plant protein. Animal protein was subdivided into protein from meat or dairy (Supplementary Table S1). Protein intake was expressed per 0.1 g/kg ideal body weight per day, per 5 g/day, and as percentage of total daily energy intake (per 2 en%). Ideal body weight was calculated by multiplying an ideal BMI of 22.5 kg/m^2 with a person's actual height (m) squared. We used ideal body weight instead of actual body weight, since normalizing protein intake to actual body weight would result in erroneously high protein requirements in overweight and obese patients.[25, 26] Total energy intake was based on energy from protein, carbohydrate and fat, but excluded alcohol.

Kidney function assessment

At baseline and 41 months follow-up, serum cystatin C (cysC) and serum creatinine (cr) were measured from stored blood samples in a central laboratory from September 1 to November 15, 2011, as previously described in detail.[27] Briefly, serum cysC was measured by a particle-enhanced immunonephelometric assay (N Latex Cystatin C, Dimension Vista 1500 Analyzer; Siemens). We used calibrators and assays of the same lot-code, which was stable (no downward drift). CysC was calibrated directly using the standard supplied by the manufacturer, traceable to the International Federation of Clinical Chemistry Working Group for Standardization of Serum Cystatin C.[28] Serum cr was measured by the modified kinetic Jaffé method (Dimension Vista 1500 Analyzer; Siemens). We calibrated directly to the standard supplied by the manufacturer from the National Institute of Standards and Technology Standard Reference Material, and postcalibration correction factor was applied.[29] We estimated glomerular filtration rate based on cystatin C ($eGFR_{cysC}$) and combined creatinine-cystatin C ($eGFR_{cr-cysC}$) at baseline and after 41 months, using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations from 2012, taking into account age, sex and race.[30] The KDIGO 2012 and NICE 2014 guidelines recommend to use $eGFR_{cysC}$ or $eGFR_{cr-cysC}$ as a confirmatory test.[10, 31] From each individual, eGFR decline or change was calculated by subtracting the eGFR at baseline from the eGFR after 41 months. Assuming a linear decline over time, we then estimated the annual kidney function decline. In the main analyses, we use $eGFR_{cysC}$ as outcome; results for $eGFR_{cr-cysC}$ are reported in Supplementary Tables S4 and S5.

Data analysis

Baseline characteristics were presented as mean with standard deviation (SD), median with interquartile range (IQR) or number (percentage), for all patients, and according to four groups of daily protein intake (<0.80, 0.80 to <1.00, 1.00 to <1.20 and ≥1.20 g/kg ideal body weight). In Supplementary Table S2 and S3, we presented baseline and dietary characteristics according to quartiles of absolute daily protein intake (g/day). The number of missing values was low: height (n=3), blood pressure (n=3), physical activity (n=9), level of education (n= 11), serum creatinine (n=76). We used multiple imputation for the main analyses to avoid bias and maintain power, using five imputations, and including all relevant baseline variables and the outcome in the model.

Linear regression was used to study the association between kidney function decline and baseline dietary intake of total protein, different types of protein (animal, plant) and protein sources (meat, dairy). All analyses were adjusted for the omega-3 fatty acid treatment groups of the Alpha Omega Trial (using 3 dummies: placebo *vs* three active treatments).[15] Further adjustments were made for the following confounders: age, sex, and total energy intake (model 1). In model 2, we additionally adjusted for alcohol consumption (g/day), cigarette smoking (current, former, never), level of education (elementary, low, moderate, high), physical activity (inactivity, low, moderate, vigorous activity) and use of renin-angiotensin system (RAS) blocking drugs. In model 3, we additionally adjusted for daily intake of saturated fat, polyunsaturated fat (PUFA), monounsaturated fat (MUFA), trans fat (g/day), dietary sodium, diabetes and systolic blood pressure. In analyses for animal protein we also adjusted for intake of plant protein and *vice versa*. Protein intake from meat was also adjusted for non-meat sources, and protein intake from dairy for non-dairy sources. In model 3, total caloric intake and all energy-providing macronutrients, except carbohydrate, were included. Therefore, in model 3 each increase in protein intake can be interpreted as a theoretical replacement of carbohydrate. In the analyses taking kidney function decline as outcome, we did not adjust for baseline eGFR, since this may lead to biased and inflated estimates.[32] To explore the presence of effect modification, analyses were repeated after stratification for age (<70 vs ≥70y), sex, CKD (eGFR <60 or ≥60 ml/min/1.73m^2), use of RAS blocking drugs, diabetes, high blood pressure (≥140/90 mmHg), or high BMI (<27 *vs* ≥27 kg/m^2). Finally, we modelled the association between total protein intake and annual $eGFR_{cysC}$ decline in a more flexible way, using restricted cubic splines with 95%-confidence intervals. The knots were chosen at the 5th, 35th, 65th, and 95th percentile of protein intake, according to general guidelines.[33]

Sensitivity analyses

First, we repeated the main analyses taking as outcome eGFR after 41 months adjusted for baseline eGFR. Second, we repeated the main analyses using as exposure daily protein intake per 0.1 g/kg actual body weight adjusted for body mass index. Third, we additionally adjusted for several micronutrients representing a healthy diet such as dietary fiber, potassium, and vitamin C. Fourth, analyses were repeated including dietary carbohydrate instead of fat intake in the substitution model. An increase in protein intake can then be interpreted as a theoretical replacement of fat. Fifth, analyses were repeated using only complete cases. Sixth, analyses were repeated after excluding patients with baseline $eGFR_{cysC}$ <30 mL/min/1.73m^2 (n=20). Finally, since blood samples were drawn after fasting or non-fasting, we additionally adjusted for fasting status (<4 hours, 4<8 hours, or ≥8 hours). Non-fasting status may have an effect on serum creatinine levels through dietary meat intake, but not on serum cystatin C level. We considered two-sided *P*-values <0.05 statistically significant. All analyses were performed using SPSS 23.0 (IBM Corp., Armonk, NY, USA), STATA Statistical Software version 14.1 (Statacorp, College Station, TX, USA), and GraphPad Prism version 7 (GraphPad Software, La Jolla, CA, USA).

RESULTS

Baseline characteristics of all patients and per category of daily protein intake (g/kg ideal body weight) are presented in Table 1. The mean age of all patients was 69 years and 80% were men. Mean $eGFR_{cysC}$ was 82 mL/min/1.73m^2 for all patients, and for patients with a daily total protein intake of <0.80 or ≥1.20 g/kg ideal body weight it was 77 mL/min/1.73m^2 and 85 mL/min/1.73m^2, respectively. Mean total protein intake was 71 g/day, providing 16% of the total energy intake, of which about 2/3 was animal and 1/3 plant protein (Table 2). The mean intake of animal protein from meat was 4 en% and from dairy it was 4 en%. For each incremental category of daily protein intake per g/kg ideal body weight, mean intake of total energy, and intake of all micronutrients and macronutrients increased (Table 2). Protein intake was highly correlated with total energy intake (Pearson correlation 0.76). Supplemental Table S2 and S3 show the baseline characteristics and dietary intake according to categories of absolute daily protein intake per g/day. Patients with a higher absolute intake of protein were more likely men, had higher height and weight, and had a higher intake if energy. Of all patients 54% used RAS blocking drugs; in patients with an $eGFR_{cysC}$ ≥90 or <60 mL/min/1.73m^2 it was 62% and 50%, respectively. About 50% of all patients persistently used RAS blocking drugs during 41 months of follow-up. Daily protein intake was similar in patients with or without RAS blocking drugs.

Table 1. Baseline characteristics of 2248 post-myocardial patients in the Alpha Omega Cohort and according to four categories of total daily protein intake.

		Total daily protein intake (g/kg ideal body weight)[¶]			
	All patients n=2248	<0.80 n=393	0.80 to <1.00 n=598	1.00 to <1.20 n=641	≥1.20 n=613
Age, y	69 ± 5	69 ± 6	69 ± 5	69 ± 5	69 ± 5
Men, no (%)	1789 (80)	302 (77)	496 (83)	512 (80)	479 (78)
Serum cystatin C, mg/L	0.97 ± 0.24	1.02 ± 0.29	0.99 ± 0.26	0.95 ± 0.22	0.93 ± 0.21
Serum creatinine,[a] mg/dL	1.02 ± 0.33	1.05 ± 0.37	1.04 ± 0.35	1.01 ± 0.30	0.98 ± 0.31
$eGFR_{cysC}$, mL/min/1.73m^2	82 ± 20	77 ± 20	80 ± 20	83 ± 19	85 ± 18
$eGFR_{cr-cysC}$, mL/min/1.73m^2	79 ± 19	75 ± 19	77 ± 19	79 ± 19	82 ± 18
Ethnicity, white, no. (%)	2222 (99)	387 (99)	590 (99)	637 (99)	606 (99)
Time since MI, y	4.0 (1.9–6.4)	4.0 (2.1–6.8)	4.0 (2.0–6.8)	4.0 (2.0–6.2)	3.9 (1.7–6.2)
High educational level,[b] no. (%)	275 (12)	34 (9)	79 (13)	90 (14)	71 (12)
Current smoker, no. (%)	352 (16)	77 (20)	109 (18)	82 (13)	84 (14)
Alcohol intake, g/day	8 (2–18)	5 (0.4–14)	9 (2–22)	8 (2–18)	8 (2–18)
Physically active,[c] no. (%)	510 (23)	84 (21)	136 (23)	137 (21)	152 (25)
Height, cm	172 ± 8	173 ± 9	173 ± 8	173 ± 8	171 ± 8
Weight, kg	82 ± 12	83 ± 13	83 ± 12	83 ± 13	81 ± 13
Body-mass index, kg/m^2	27.6 ± 3.6	27.6 ± 3.6	27.4 ± 3.5	27.7 ± 3.6	27.8 ± 3.7
≥30 kg/m^2, no. (%)	506 (23)	81 (21)	125 (21)	149 (23)	151 (25)
High blood pressure,[d] no. (%)	1275 (57)	225 (57)	344 (58)	367 (57)	338 (55)

Table 1. Continued

Systolic BP, mmHg	144 ± 21	144 ± 22	144 ± 21	145 ± 22	142 ± 20
Diastolic BP, mmHg	82 ± 11	82 ± 11	82 ± 11	82 ± 11	81 ± 10
BP-lowering drugs,[e] no. (%)	1954 (87)	354 (90)	522 (87)	539 (84)	537 (88)
RAS blocking drugs[f]	1222 (54)	205 (52)	335 (56)	333 (52)	349 (57)
Plasma glucose,[g] mg/dL	6.0 ± 1.9	6.0 ± 1.8	6.0 ± 1.9	6.0 ± 1.8	6.1 ± 2.1
Diabetes,[h] no. (%)	405 (18)	72 (18)	109 (18)	108 (17)	115 (19)
Glucose-lowering drugs,[e] no. (%)	289 (13)	56 (14)	72 (12)	79 (12)	81 (13)
Serum LDL,[i] mg/dL	2.7 ± 0.8	2.7 ± 0.9	2.7 ± 0.8	2.7 ± 0.8	2.7 ± 0.7
Lipid-modifying drugs,[e] no. (%)	1944 (87)	345 (88)	509 (85)	561 (88)	528 (86)
Anti-thrombotic drugs,[e] no. (%)	2201 (98)	383 (98)	582 (97)	628 (98)	606 (99)

RAS, renin-angiotensin system; BP, blood pressure; cr, creatinine; cysC, cystatin C; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein; MI, myocardial infarction.
Data are reported as number of patients (%), mean ± SD or median (interquartile range).
¶ From 3 patients with missing height, no intake in g/kg ideal body weight could be calculated, hence numbers from the four categories do not add up to 2248.
[a] To convert the values for creatinine to µmol/L multiply by 88.40.
[b] Higher vocational education or university.
[c] Defined as ≥3 Metabolic Equivalent of Tasks (MET) for ≥30 minutes per day during ≥5 days/week.
[d] Defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg.
[e] Blood pressure-lowering drugs ATC codes C02, C03, C07, C08, and C09. Glucose-lowering drugs ATC codes A10, A10A, A10B, A10X. Lipid-modifying drugs ATC codes C10, C10AA. Antithrombotic drugs ATC code B01.
[f] Defined as ATC code C09, renin-angiotensin system inhibitors.
[g] Non-fasting; to convert the values for glucose to mg/dL, divide by 0.05551.
[h] Self-reported diagnosis by a physician, use of glucose-lowering drugs, or hyperglycemia.
[i] Non-fasting; to convert the values for LDL-cholesterol to mg/dL, divide by 0.02586.

Table 2. Dietary intake of macronutrients and micronutrients of 2248 post-myocardial patients of the Alpha Omega Cohort and according to four categories of daily total protein intake.

			Total daily protein intake (g/kg ideal body weight)¶			
		All patients n=2248	<0.80 n=393	0.80 to <1.00 n=598	1.00 to <1.20 n=641	≥1.20 n=613
Total energy[a]	kcal/day	1827 ± 497	1346 ± 316	1659 ± 364	1874 ± 359	2250 ± 469
Total protein	g/day	71 ± 19	46 ± 8	61 ± 6	73 ± 8	92 ± 14
	en%	16 ± 3	14 ± 3	15 ± 3	16 ± 3	17 ± 3
Animal protein	g/day	43 ± 15	25 ± 8	36 ± 7	45 ± 8	60 ± 12
	en%	10 ± 3	8 ± 3	9 ± 3	10 ± 3	11 ± 3
From meat	g/day	17 ± 9	9 ± 7	15 ± 7	18 ± 7	22 ± 8
	en%	4 ± 2	3 ± 2	4 ± 2	4 ± 2	4 ± 2
From dairy	g/day	18 ± 10	10 ± 5	14 ± 7	18 ± 8	27 ± 12
	en%	4 ± 2	3 ± 2	3 ± 2	4 ± 2	5 ± 2
Plant protein	g/day	27 ± 8	21 ± 5	25 ± 6	28 ± 6	33 ± 8
	en%	6 ± 1	6 ± 1	6 ± 1	6 ± 1	6 ± 1
Total	g/day	223 ± 68	173 ± 49	204 ± 57	228 ± 58	268 ± 68
carbohydrate	en%	49 ±7	51 ± 8	49 ± 7	48 ± 7	48 ± 6
Total fat	g/day	73 ± 27	52 ± 20	66 ± 23	75 ± 22	90 ± 27
	en%	35 ± 7	35 ± 8	36 ± 7	36 ± 7	36 ± 6
Fiber	g/day	22 ± 7	17 ± 5	20 ± 5	22 ± 6	26 ± 7
Sodium[b]	mg/day	2217 ± 661	1541 ± 371	1950 ± 403	2276 ± 463	2849 ± 602
Potassium	mg/day	3259 ± 851	2438 ± 570	2936 ± 576	3344 ± 613	4007 ± 791
Vitamin C	mg/day	97 ± 54	75 ± 41	87 ± 51	103 ± 58	116 ± 53

en%, percentage of total energy intake.
¶ From 3 patients with missing height, no intake in g/kg ideal body weight could be calculated, hence numbers from the four categories do not add up to 2248.
Animal protein from meat and dairy do not add up to total animal protein, because total animal protein from also includes protein from eggs and fish.
[a] Excluding calories from alcohol.
[b] Only from foods, to convert to intake of salt (sodium chloride) multiply by 2.5.

Protein intake and annual kidney function decline

For all patients the mean (95%-CI) annual change in $eGFR_{cysC}$ and $eGFR_{cr-cysC}$ was -1.30 (-1.43; -1.17) and -1.71 (-1.87; -1.56) mL/min/1.73m^2, respectively. Total protein intake was inversely associated with annual kidney function decline. The fully adjusted model showed that the annual change in $eGFR_{cysC}$ was doubled in patients with a daily total protein intake >1.20 compared to <0.80 g/kg ideal body weight: -1.60 (-1.92; -1.28) compared to -0.84 (-1.21; -0.46) mL/min/1.73m^2 (Table 3). Comparable associations were observed for $eGFR_{cr-cysC}$ (Supplementary Table S4). Restricted cubic spline analysis confirmed a strong linear association between protein intake and annual kidney function decline (Figure 1). We also found an inverse association between the intake of animal protein and both $eGFR_{cysC}$ or $eGFR_{cr-cysC}$, and a similar but non-significant association for plant protein (Table 4 and Supplementary Table S5). Compared to animal protein from meat, higher dairy protein intake was associated with a slower kidney function decline (Table 4). Each extra 0.1 g/kg ideal body weight daily intake of animal protein from meat or dairy was associated with an additional $eGFR_{cysC}$ decline of -0.14 (-0.25; -0.03) and -0.06 (-0.16; 0.04) mL/min.1.73m^2, respectively (Table 4). Taking $eGFR_{cr-cysC}$ as outcome, the associations with protein from dairy and meat were comparable (Supplementary Table S5). Results remained similar when daily protein intake was expressed per 5 g/day or per 2 en%. We found no evidence for effect modification with regard to kidney function decline between protein intake and pre-defined factors, except the association between protein intake and eGFR decline was stronger for patients with compared to without diabetes (Figure 2). Finally, with increasing protein intake, we observed no difference in annual $eGFR_{cysC}$ decline between patients persistently using RAS blocking drugs and nonusers.

Table 3: Annual eGFR change, based on serum cystatin C, according to daily total protein intake in 2248 post-myocardial patients of the Alpha Omega Cohort.

		Total daily protein intake (g/kg ideal body weight)				P trend
		<0.80 n = 393	0.80 to <1.00 n = 599	1.00 to <1.20 n = 643	≥1.20 n = 613	
Annual $eGFR_{cysC}$ change	Crude	-1.17 (-1.48; -0.85)	-1.28 (-1.54; -1.03)	-1.44 (-1.68; -1.19)	-1.26 (-1.51; -1.01)	0.5
(mL/min/1.73m^2)	Model 1	-0.79 (-1.15; -0.44)	-1.12 (-1.38; -0.86)	-1.47 (-1.71; -1.23)	-1.63 (-1.93; -1.34)	<0.001
	Model 2	-0.79 (-1.14; -0.43)	-1.10 (-1.36; -0.84)	-1.50 (-1.74; -1.26)	-1.62 (-1.91; -1.33)	<0.001
	Model 3	-0.84 (-1.21; -0.46)	-1.10 (-1.37; -0.84)	-1.48 (-1.72; -1.24)	-1.60 (-1.92; -1.28)	0.003

$eGFR_{cysC}$, cystatin C based estimated glomerular filtration rate
Model 1: adjusted for age, sex, and total energy intake.
Model 2: Model 1 plus additional adjustment for education, alcohol, smoking, physical activity, RAS blocking drugs.
Model 3: Model 2 plus additional adjustment for intake of fat (mono- and poly-unsaturated fat, saturated fat, and trans fat), dietary sodium, diabetes, and systolic blood pressure.

Table 4: Annual change in eGFR (mL/min/1.73m²), based on serum cystatin C, per unit increment daily intake of total, animal, or plant-based protein in 2248 post-myocardial patients of the Alpha Omega Cohort.

Unit		Total protein	Animal protein			Plant protein
			Total	From meat	From dairy	
0.1 g/kg ideal body weight	Crude	-0.01 (-0.05; 0.04)	-0.03 (-0.09; 0.03)	-0.09 (-0.19; 0.02)	0.02 (-0.06; 0.10)	0.08 (-0.03; 0.20)
	Model 1	-0.12 (-0.18; -0.05)**	-0.12 (-0.19; -0.05)**	-0.15 (-0.25; -0.05)*	-0.05 (-0.14; 0.05)	-0.04 (-0.20; 0.13)
	Model 2	-0.12 (-0.18; -0.05)**	-0.11 (-0.18; -0.04)*	-0.13 (-0.23; -0.03)*	-0.05 (-0.14; 0.04)	-0.06 (-0.23; 0.10)
	Model 3	-0.12 (-0.19; -0.04)*	-0.12 (-0.19; -0.04)*	-0.14 (-0.25; -0.03)*	-0.06 (-0.16; 0.04)	-0.12 (-0.32; 0.07)
5 g	Crude	-0.01 (-0.04; 0.03)	-0.02 (-0.07; 0.02)	-0.07 (-0.14; 0.01)	0.01 (-0.05; 0.07)	0.06 (-0.03; 0.15)
	Model 1	-0.09 (-0.15; -0.04)*	-0.09 (-0.14; -0.04)*	-0.11 (-0.19; -0.03)*	-0.03 (-0.10; 0.04)	0.01 (-0.12; 0.14)
	Model 2	-0.09 (-0.15; -0.04)*	-0.08 (-0.14; -0.03)*	-0.10 (-0.18; -0.02)*	-0.04 (-0.11; 0.04)	-0.02 (-0.15; 0.12)
	Model 3	-0.09 (-0.16; -0.02)*	-0.09 (-0.16; -0.02)*	-0.11 (-0.20; -0.02)*	-0.05 (-0.13; 0.03)	-0.10 (-0.29; 0.09)
2 en%	Crude	-0.17 (-0.26; -0.08)**	-0.16 (-0.25; -0.07)**	-0.20 (-0.33; -0.06)	-0.04 (-0.17; 0.09)	-0.04 (-0.27; 0.19)
	Model 1	-0.19 (-0.29; -0.10)**	-0.18 (-0.28; -0.09)**	-0.21 (-0.34; -0.07)*	-0.07 (-0.20; 0.06)	0.04 (-0.21; 0.28)
	Model 2	-0.19 (-0.29; -0.09)**	-0.18 (-0.27; -0.08)**	-0.19 (-0.32; -0.05)*	-0.08 (-0.21; 0.05)	-0.01 (-0.26; 0.24)
	Model 3	-0.20 (-0.31; -0.08)**	-0.20 (-0.31; -0.08)*	-0.22 (-0.37; -0.07)*	-0.11 (-0.27; 0.04)	-0.20 (-0.55; 0.14)

en%, percentage of total energy intake.
*p<0.05 **p<0.001
Model 1: adjusted for age, sex, and total energy intake
Model 2: Model 1 plus additional adjustment for education, alcohol, smoking, physical activity, RAS blocking drugs.
Model 3: Model 2 plus additional adjustment for intake of fat (mono- and poly-unsaturated fat, saturated fat, and trans fat), dietary sodium, diabetes, and systolic blood pressure; animal protein was also adjusted for plant protein, and vice versa.

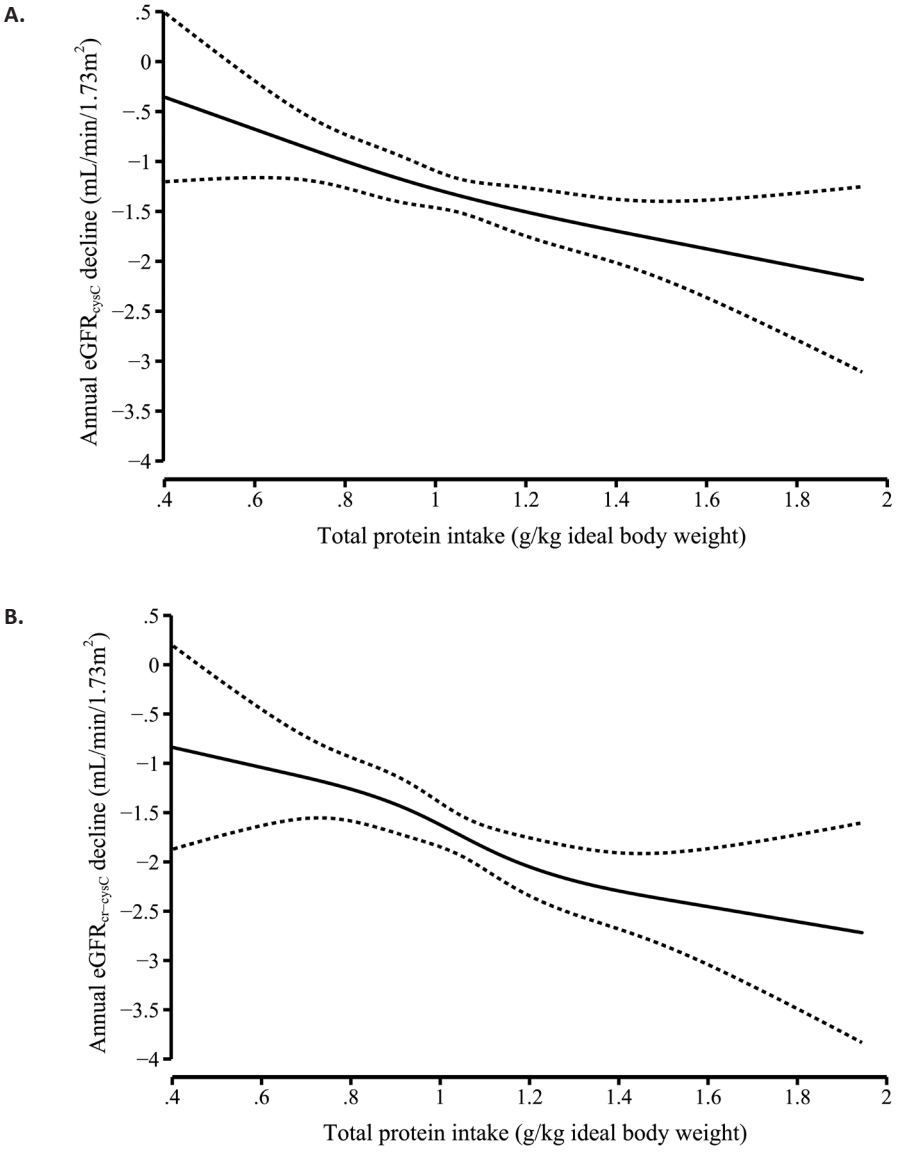

Figure 1: Association (with 95%-confidence interval) between daily total protein intake (g/kg ideal body weight) and annual cystatin C based (A) and creatinine-cystatin C based (B) eGFR. Modelled by restricted cubic splines with knots at the 5th, 35th, 65th, and 95th percentile of protein intake. In these analyses patients with a daily protein intake ≤0.4 (n=6) or >2.0 (n=11) g/kg ideal body weight were excluded. The model was adjusted for age, sex, total energy intake, education, alcohol, smoking, physical activity, RAS blocking drugs, intake of fat (mono- and poly-unsaturated fat, saturated fat, and trans fat), dietary sodium, diabetes, and systolic blood pressure. eGFR, estimated glomerular filtration rate

Sensitivity analyses

Taking as outcome eGFR after 41 months of follow-up adjusted for baseline eGFR (data not shown), or daily protein intake per 0.1 g/kg actual body weight adjusted for body mass index, yielded similar results (Supplementary Table S6). Additional adjustment for dietary fiber, potassium, and vitamin C yielded slightly stronger effect estimates. Results remained similar when replacing protein in the model by fat instead of carbohydrates. Type of fat, saturated or unsaturated, did not affect the results. Additional adjustment for fasting status did not change our results. Finally, results remained essentially unchanged analyzing complete cases only, or excluding patients with baseline eGFR <30 mL/min/1.73m^2.

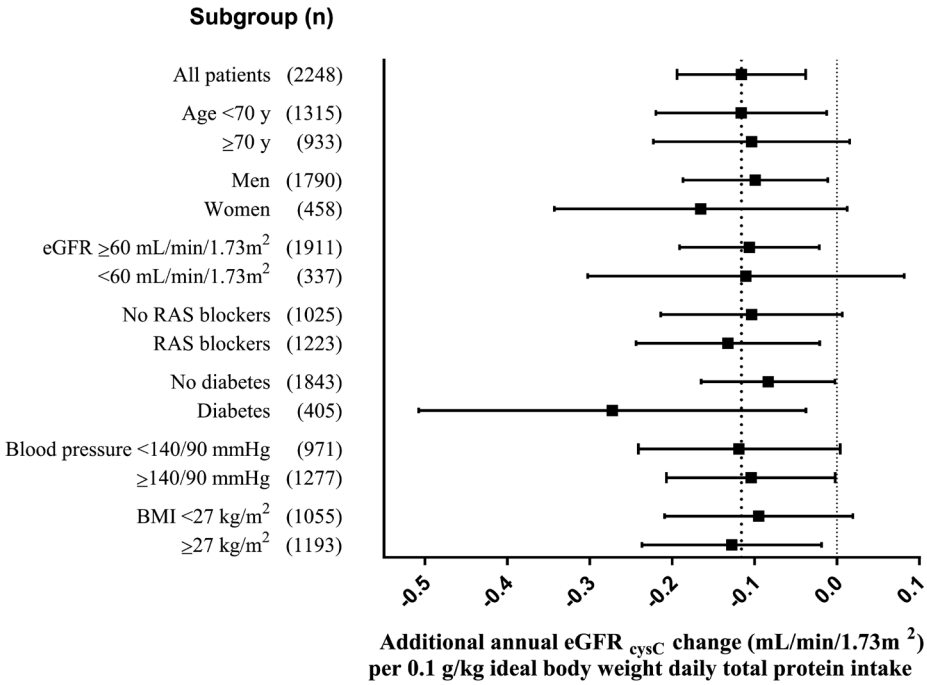

Figure 2: Additional annual change in eGFR$_{cysC}$ per 0.1 g/kg ideal body weight increased daily total protein intake, according to different subgroups. The model was fully adjusted (model 3) for age, sex, total energy intake, education, alcohol, smoking, physical activity, RAS blocking drugs, for intake of fat (mono- and poly-unsaturated fat, saturated fat, and trans fat), dietary sodium, diabetes, and systolic blood pressure. BMI, body mass index; eGFR, estimated glomerular filtration rate; RAS, renin-angiotensin system.

DISCUSSION

This is the first and largest cohort of older state-of-the-art drug-treated post-MI patients showing that high protein intake is associated with accelerated kidney function decline. Patients with a daily total protein intake of ≥1.20 compared to <0.80 g/kg ideal body weight had a 2-fold greater rate of annual kidney function decline of -1.60 versus -0.84 mL/min/1.73m^2. Each extra daily protein intake of 0.1 g/kg ideal body weight was associated with an additional kidney function decline of -0.12 mL/min/1.73m^2 per year. The associations of total, animal or plant protein with kidney function decline were comparable.

Our findings are in line with the current KDIGO guidelines recommending to avoid a daily total protein intake higher than 1.30 g/kg ideal body weight and restrict protein intake to 0.80 g/kg for patients with diabetes and those at risk for CKD.[10] Current guidelines make no recommendations with regard to animal and plant protein intake. However, for low protein diets it is recommended that about half consists of "high biologic value" animal protein, such as dairy or meat, to ensure a sufficient daily intake of essential amino acids.[11, 34] For healthy individuals the recommended dietary allowance for protein is 0.80 g/kg per day. To prevent protein wasting more than 10% of daily energy intake should be derived from protein.[35] We showed that post-MI patients with a daily protein intake of <0.80 g/kg ideal body weight, which on average represents about 14% of the total energy intake, had the lowest annual $eGFR_{cysC}$ decline of -0.84 mL/min/1.73m^2. The mean (95% CI) annual eGFR decline of -1.3 (-1.4 to -1.2) mL/min/1.73m^2 in our study is lower than the -2.2 (-5.0 to -0.9) mL/min/1.73m^2 in post-MI patients reported in the Prevention of Renal and Vascular End-stage Disease (PREVEND) study.[4] The slower rate of kidney function decline in our study can be explained by more stringent guidelines on secondary prevention of cardiovascular disease during the Alpha Omega Trial (2002 to 2009) than the PREVEND study (1997 to 2005), and the more precise estimate of the kidney function decline given the smaller 95% CI of our study, as we previously discussed in more detail.[36] In our cohort of post-MI patients, the total energy intake differs substantially between the lowest and highest category of protein intake. This is explained by the high correlation between protein intake and energy intake (Pearson correlation 0.76), and a similar trend was shown in 11,952 individuals of the Atherosclerosis Risk in Communities study.[37] The low absolute intake of total energy in the lowest category of protein intake, may partly be explained by measurement error.[38] Therefore, it is important to adjust in the model for energy intake to reduce the influence of measurement error and control for extraneous variation.[39]

Only few studies, mostly population-based, investigated the association between total protein intake and kidney function decline. The Singapore Chinese Health Study showed in middle-aged individuals a 20% greater risk of end-stage renal disease for the highest three compared to lowest quartile of total protein intake, over a mean follow-up of 15 years.[40] Unfortunately, information on baseline eGFR was not available in this cohort. Others found in middle-aged women (eGFR 55-80 mL/min/1.73m^2) that each incremental 10 gram of daily total protein intake was associated with an additional eGFR decline of -1.69 mL/min/1.73m^2 after 11 years of follow-up.[41] In contrast, total protein intake was not associated with CKD risk in the Doetinchem study, a Dutch community-based cohort, as well as in two US community-based cohorts.[37, 42, 43] Compared to Alpha Omega Cohort, participants in these three aforementioned cohorts were about 20 years younger, had a normal creatinine-based eGFR, and had less comorbidities.

We observed in the present study, that the magnitude of the associations did not differ for animal and plant protein with regards to kidney function decline in older post-MI patients. The population-based Doetinchem study found no association for either animal or plant protein intake with kidney function decline.[43] The ARIC study, a US cohort of middle-aged individuals without cardiovascular disease and normal kidney function, found no association between the intake of animal protein and kidney function. However, they showed a 24% lower risk of CKD in individuals in the highest compared to lowest quintile of plant protein intake.[37]

We found a twice as low association of dairy compared to meat protein intake with kidney function decline in elderly post-MI patients. In contrast, the ARIC study showed that individuals in the highest compared to lowest quintile of low-fat dairy intake had a 20% lower CKD risk.[37] In the Doetinchem study, individuals in the highest compared to lowest tertile of total dairy intake had a 0.2 mL/min/1.73m^2 slower annual kidney function decline.[43] As opposed to the present study, the ARIC and Doetinchem study did not analyze the effect of protein from dairy, but from dairy foods as a whole.

Several mechanisms may explain the association of protein intake with accelerated kidney function decline. A high-protein diet dilates the glomerular afferent arteriole, resulting in hyperfiltration and subsequent glomerular damage owing to inflammation and fibrosis.[44] In contrast, a low-protein diet lowers the intraglomerular pressure, a beneficial effect that is enhanced if combined with RAS blockers that dilate the efferent arteriole.[45, 46] We observed comparable associations of animal and plant protein intake regarding the rate of kidney function decline. The strongest kidney function decline was observed for meat and plant protein, whereas for dairy protein the decline was only half

compared with meat and plant protein. However, the latter association was not significant. More research is needed to determine whether or not dairy protein is superior to meat and plant protein with regard to slowing down kidney function decline. Subgroup analyses showed a three-fold stronger association between protein intake and eGFR decline in patients with compared to without diabetes. Diabetes increases the risk of glomerular hyperfiltration and proteinuria, possibly leading to higher susceptibility to the detrimental effects of a high protein diet in these patients.[47] Our results suggest that a low-protein diet may be especially beneficial for patients with diabetes to slow down kidney function decline. However, confidence intervals were broad, and results should be interpreted with caution.

This study has several limitations. First, the observational study design prevents causal inference. Second, despite extensive adjustments we cannot rule out residual confounding. Protein is not consumed in isolation but as part of a dietary pattern, composed of numerous nutrients and bio-actives of which each may have its own effects on kidney function.[48] Therefore, it is difficult to attribute any observed effect solely to the protein content or source. Third, we estimated kidney function decline using only one measurement at two time points, which may reduce precision. If anything, this may have resulted in underestimation of the association between protein intake and kidney function decline. Fourth, we had no information on proteinuria, an important risk factor for kidney function decline. Fifth, dietary data were obtained by FFQs, which may under- or overestimate the absolute protein intake.[38] The modified FFQ that we used was not validated, however it was an extended version of a previously biomarker-validated FFQ, including more detailed questions about food consumption.[21, 22] Dietary protein intake was assessed at baseline, and we did not take into account changes of intake during follow-up. However, previous studies showed that the dietary pattern remained stable, especially at older age, over a timespan up to seven years.[24] Sixth, we had no information on biomarkers like urinary urea nitrogen, to validate protein intake obtained from the FFQ. Furthermore, about 8% of patients died during follow-up and were, therefore, not included in the analyses. However, intake of protein and other macro-nutrients was similar for patients included in the current analyses compared to patients who died during follow-up (not shown), which makes selection bias unlikely. Finally, this cohort consisted of post-MI patients, which may limit generalizability to other populations.

Our prospective analysis has also several strengths. First, we estimated kidney function based on two different endogenous markers. Second, we measured cystatin C, which is currently the most accurate marker for kidney function, and is not influenced by glomerular hyperfiltration.[10, 49, 50] Moreover,

serum cystatin C is, in contrast to creatinine, not influenced by dietary meat intake and muscle mass.[51–54] Third, we used different measures of protein intake: the absolute protein intake in g/day, intake expressed in % of energy, and the intake adjusted for ideal body weight. Each approach led to similar conclusions. Finally, we used substitution models since the association between kidney function decline does not only depend on the macro-nutrient of interest, namely protein, but also the replacement of other macro-nutrients, such as carbohydrates or fat.[55]

In conclusion, we found that a higher dietary intake of total protein was associated with a more rapid loss of kidney function in older post-MI patients. Despite the fact that our patients received state-of-the-art drug treatment, we observed a beneficial effect of a low-protein intake on kidney function.

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DISCLOSURES

EH is a member of the Guideline Committee of the Dutch Federation of Nephrology. JG received research funding from Unilever R&D for epidemiological studies of dietary fatty acids and is a member of the Standing Committee on Nutrition of the Dutch Health Council, Working Group on Minerals of the European Food and Safety Authority, and Dutch Academy for Nutritional Sciences, and is a Fellow of the American Heart Association. DK received research funding from the Royal Netherlands Academy of Arts and Sciences and is Member of the Dutch Academy of Nutritional Sciences. KE and JF report that they have no disclosures.

AUTHORS' CONTRIBUTIONS

Research idea and study design: EH, KE, JG, DK; data acquisition: DK, JG, EH; data analysis/interpretation: KE, EH, JG, DK, JF; statistical analysis: KE, EH; supervision and mentorship: EH, JF, JG. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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SUPPLEMENTARY DATA

Table S1: types of food contributing to total intake of meat or dairy.

Protein source	Included food types
Meat	Beef, calf, pork, chicken, duck, turkey, pheasant, partridge, horse, rabbit, hare, sheep, lamb, roe, cooked liver, liver- or kidney products, sausage, bacon, minced meat, hamburger, snacks, pate, ham, other meat
Dairy	All cheese products (20+, 30+, high fat and low fat cheese, other cheese), milk and chocolate milk (full, semi-skimmed, skim), buttermilk, yoghurt (full, semi-skimmed, skim), whipped cream, coffee milk or cream, creamer, other milk products

Table S2. Baseline characteristics of 2248 post-myocardial patients in the Alpha Omega Cohort and according to quartiles of total daily protein intake (g/day).

		Total protein intake (g/day)			
	All patients n=2248	21.2 to <58.1 n=562	58.1 to <69.1 n=562	69.1 to <81.5 n=562	81.5 to 146.8 n=562
Age, y	69 ± 5	69 ± 5	69 ± 5	69 ± 5	68 ± 5
Men, no (%)	1789 (80)	379 (67)	439 (78)	470 (84)	502 (89)
Serum cystatin C, mg/L	0.97 ± 0.24	1.02 ± 0.30	0.97 ± 0.23	0.95 ± 0.23	0.93 ± 0.20
Serum creatinine,[a] mg/dL	1.02 ± 0.33	1.04 ± 0.40	1.02 ± 0.30	1.02 ± 0.32	0.99 ± 0.30
$eGFR_{cysC}$, mL/min/1.73m^2	82 ± 20	77 ± 21	81 ± 19	84 ± 19	85 ± 18
$eGFR_{cr\text{-}cysC}$, mL/min/1.73m^2	79 ± 19	74 ± 20	78 ± 18	80 ± 19	82 ± 17
Ethnicity, white, no. (%)	2222 (99)	551 (98)	555 (99)	560 (99.6)	557 (99)
Time since MI, y	4.0 (1.9–6.4)	4.2 (2.1–6.8)	4.0 (2.0–6.5)	3.9 (2.1–6.3)	3.9 (1.7–6.2)
High educational level,[b] no. (%)	275 (12)	39 (7)	75 (13)	79 (14)	82 (15)
Current smoker, no. (%)	352 (16)	110 (20)	88 (16)	78 (14)	76 (14)
Alcohol intake, g/day	8 (2–18)	5 (1–13)	8 (2–19)	9 (2–21)	9 (3–20)
Physically active,[c] no. (%)	510 (23)	111 (20)	131 (23)	127 (23)	141 (25)
Height, cm	172 ± 8	170 ± 9	172 ± 8	173 ± 8	174 ± 8
Weight, kg	82 ± 12	80 ± 12	81 ± 12	83 ± 12	84 ± 12
Body-mass index, kg/m^2	27.6 ± 3.6	27.8 ± 3.8	27.4 ± 3.7	27.7 ± 3.5	27.6 ± 3.5
≥30 kg/m^2, no. (%)	506 (23)	140 (25)	114 (20)	130 (23)	122 (22)
High blood pressure,[d] no. (%)	1275 (57)	322 (57)	314 (56)	326 (58)	313 (56)

Table S2. Continued

Systolic BP, mmHg	144 ± 21	144 ± 22	144 ± 21	144 ± 21	142 ± 21
Diastolic BP, mmHg	82 ± 11	81 ± 11	82 ± 11	82 ± 11	81 ± 10
BP-lowering drugs,[e] no. (%)	1954 (87)	505 (90)	476 (85)	475 (85)	499 (89)
RAS blocking drugs[f]	1222 (54)	304 (54)	291 (52)	303 (54)	325 (58)
Plasma glucose,[g] mg/dL	6.0 ± 1.9	6.1 ± 2.0	5.9 ± 1.8	6.1 ± 1.9	6.0 ± 2.0
Diabetes,[h] no. (%)	405 (18)	113 (20)	98 (17)	98 (17)	96 (17)
Glucose-lowering drugs,[e] no. (%)	289 (13)	84 (15)	65 (12)	72 (13)	68 (12)
Serum LDL,[i] mg/dL	2.7 ± 0.8	2.8 ± 0.9	2.7 ± 0.8	2.7 ± 0.8	2.7 ± 0.7
Lipid-modifying drugs,[e] no. (%)	1944 (87)	488 (87)	480 (85)	490 (87)	487 (87)
Anti-thrombotic drugs,[e] no. (%)	2201 (98)	545 (97)	545 (97)	556 (99)	556 (99)

RAS, renin-angiotensin system; BP, blood pressure; cr, creatinine; cysC, cystatin C; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein; MI, myocardial infarction.
Data are reported as number of patients (%), mean ± SD or median (interquartile range).
[a] To convert the values for creatinine to µmol/L multiply by 88.40.
[b] Higher vocational education or university.
[c] Defined as ≥3 Metabolic Equivalent of Tasks (MET) during ≥5 days/week.
[d] Defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg.
[e] Blood pressure-lowering drugs ATC codes C02, C03, C07, C08, and C09. Glucose-lowering drugs ATC codes A10, A10A, A10B, A10X. Lipid-modifying drugs ATC codes C10, C10AA. Antithrombotic drugs ATC code B01.
[f] Defined as ATC code C09, renin-angiotensin system inhibitors.
[g] Non-fasting; to convert the values for glucose to mg/dL, divide by 0.05551.
[h] Self-reported diagnosis by a physician, use of glucose-lowering drugs, or hyperglycemia.
[i] Non-fasting; to convert the values for LDL-cholesterol to mg/dL, divide by 0.02586.

Table S3. Dietary intake of macronutrients and micronutrients of 2248 post-myocardial patients of the Alpha Omega Cohort and according to quartiles of daily total protein intake (g/day).

			Total protein intake (g/day)			
		All patients n=2248	21.2 to <58.1 n=562	58.1 to <69.1 n=562	69.1 to <81.5 n=562	81.5 to 146.8 n=562
Total energy[a]	kcal/day	1827 ± 497	1370 ± 318	1685 ± 309	1924 ± 329	2327 ± 439
Total protein	g/day	71 ± 19	48 ± 8	64 ± 3	75 ± 4	95 ± 12
	en%	16 ± 3	15 ± 3	16 ± 3	16 ± 3	17 ± 3
Animal protein	g/day	43 ± 15	27 ± 8	38 ± 6	47 ± 6	61 ± 11
	en%	10 ± 3	8 ± 3	10 ± 3	10 ± 3	11 ± 3
From meat	g/day	17 ± 9	10 ± 7	15 ± 7	18 ± 7	23 ± 8
	en%	4 ± 2	3 ± 2	4 ± 2	4 ± 2	4 ± 2
From dairy	g/day	18 ± 10	10 ± 5	15 ± 7	18 ± 8	27 ± 12
	en%	4 ± 2	3 ± 2	4 ± 2	4 ± 2	5 ± 2
Plant protein	g/day	27 ± 8	21 ± 5	25 ± 6	29 ± 6	34 ± 7
	en%	6 ± 1	6 ± 1	6 ± 1	6 ± 1	6 ± 1
Total carbohydrate	g/day	223 ± 68	174 ± 51	207 ± 52	234 ± 55	276 ± 66
	en%	49 ±7	51 ± 8	49 ± 7	48 ± 7	47 ± 6
Total fat	g/day	73 ± 27	53 ± 20	67 ± 21	76 ± 21	94 ± 26
	en%	35 ± 7	35 ± 8	36 ± 7	36 ± 6	36 ± 6
Fiber	g/day	22 ± 7	17 ± 6	20 ± 5	23 ± 5	27 ± 7
Sodium[b]	mg/day	2217 ± 661	1599 ± 383	1988 ± 383	2340 ± 417	2942 ± 551
Potassium	mg/day	3259 ± 851	2494 ± 584	3022 ± 516	3427 ± 586	4092 ± 766
Vitamin C	mg/day	97 ± 54	77 ± 44	91 ± 51	105 ± 58	117 ± 54

en%, percentage of total energy intake.
Animal protein from meat and dairy do not add up to total animal protein, because total animal protein from also includes protein from eggs and fish.
[a] Excluding calories from alcohol.

Table S4: Annual eGFR change, based on serum creatinine-cystatin C, according to daily total protein intake in 2248 post-myocardial patients of the Alpha Omega Cohort.

		Total protein intake (g/kg ideal body weight)				
		<0.80 n = 393	0.80 to <1.00 n = 599	1.00 to <1.20 n = 643	≥1.20 n = 613	P trend
Annual $eGFR_{cr\text{-}cysC}$ change	Crude	-1.62 (-1.99; -1.26)	-1.62 (-1.92; -1.33)	-1.77 (-2.06; -1.48)	-1.80 (-2.10; -1.51)	0.3
(mL/min/1.73m^2)	Model 1	-1.28 (-1.69; -0.86)	-1.50 (-1.80; -1.19)	-1.80 (-2.09; -1.51)	-2.12 (-2.46; -1.78)	0.003
	Model 2	-1.29 (-1.70; -0.88)	-1.48 (-1.79; -1.18)	-1.82 (-2.11; -1.53)	-2.10 (-2.44; -1.75)	0.004
	Model 3	-1.22 (-1.65; -0.78)	-1.43 (-1.74; -1.13)	-1.81 (-2.10; -1.52)	-2.21 (-2.58; -1.83)	0.002

$eGFR_{cr\text{-}cysC}$, combined creatinine and cystatin C based estimated glomerular filtration rate
Model 1: adjusted for total caloric intake, age, sex, and total energy intake.
Model 2: Model 1 plus additional adjustment for education, alcohol, smoking, physical activity, RAS blocking drugs.
Model 3: Model 2 plus additional adjustment for intake of fat (mono- and poly-unsaturated fat, saturated fat, and trans fat), dietary sodium, diabetes, and systolic blood pressure.

Table S5: Annual change in eGFR (mL/min/1.73m²), based on serum creatinine-cystatin C (B), per unit increment daily intake of total, animal, or plant-based protein in 2248 post-myocardial patients of the Alpha Omega Cohort.

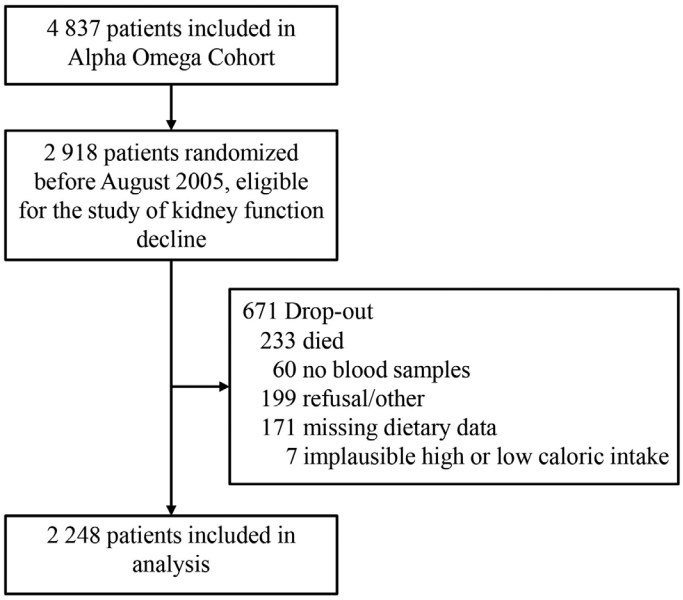
Unit		Total protein	Animal protein			Plant protein
			Total	From meat	From dairy	
0.1 g/kg ideal	Crude	-0.02 (-0.07; 0.04)	-0.05 (-0.12; 0.02)	-0.07 (-0.18; 0.05)	-0.05 (-0.15; 0.5)	0.10 (-0.03; 0.24)
body weight	Model 1	-0.11 (-0.19; -0.03)*	-0.12 (-0.20; -0.04)*	-0.11 (-0.23; 0.01)	-0.10 (-0.21; 0.1)	0.03 (-0.16; 0.22)
	Model 2	-0.11 (-0.18; -0.03)*	-0.11 (-0.19; -0.03)*	-0.09 (-0.21; 0.03)	-0.10 (-0.21; 0.003)	-0.00 (-0.19; 0.19)
	Model 3	-0.15 (-0.24; -0.06)*	-0.15 (-0.23; -0.06)*	-0.15 (-0.28; -0.01)*	-0.14 (-0.5; -0.02)*	-0.14 (-0.37; 0.10)
5 g/day	Crude	-0.003 (-0.04; 0.04)	-0.03 (-0.08; 0.02)	-0.04 (-0.13; 0.05)	-0.04 (-0.11; 0.04)	0.10 (-0.001; 0.20)
	Model 1	-0.09 (-0.16; -0.03)*	-0.10 (-0.16; -0.03)*	-0.09 (-0.18; 0.01)	-0.08 (-0.16; 00003)	0.05 (-0.10; 0.20)
	Model 2	-0.09 (-0.16; -0.03)*	-0.09 (-0.15; -0.03)*	-0.07 (-0.16; 0.02)	-0.08 (-0.17; -0.002)*	0.03 (-0.13; 0.18)
	Model 3	-0.12 (-0.20; -0.04)*	-0.12 (-0.20; -0.04)*	-0.11 (-0.21; -0.01)*	-0.11 (-0.21; -0.01)*	-0.10 (-0.32; 0.12)
2 en%	Crude	-0.19 (-0.29; -0.08)**	-0.18 (-0.29; -0.08)**	-0.14 (-0.30; 0.01)	-0.17 (-0.32; -0.02)*	-0.03 (-0.30; 0.24)
	Model 1	-0.19 (-0.30; -0.07)*	-0.18 (-0.29; -0.07)*	-0.13 (-0.29; 0.02)	-0.17 (-0.32; -0.01)*	0.05 (-0.23; 0.33)
	Model 2	-0.18 (-0.29; -0.07)*	-0.17 (-0.28; -0.06)*	-0.11 (-0.27; 0.05)	-0.18 (-0.33; -0.02)*	0.002 (-0.31; 0.32)
	Model 3	-0.23 (-0.37; -0.10)**	-0.23 (-0.37; -0.09)**	-0.20 (-0.37; -0.02)*	-0.24 (-0.43; -0.06)*	-0.27 (-0.67; 0.14)

en%, percentage of total energy intake.
*p<0.05 **p<0.001
Model 1: adjusted for, age, sex, and total energy intake
Model 2: Model 1 plus additional adjustment for education, alcohol, smoking, physical activity, RAS blocking drugs.
Model 3: Model 2 plus additional adjustment for intake of fat (mono- and poly-unsaturated fat, saturated fat, and trans fat), dietary sodium, diabetes, and systolic blood pressure; animal protein was also adjusted for plant protein, and vice versa.

Table S6: Annual change in eGFR (mL/min/1.73m²), based on serum cystatin C, per incremental 0.1 g/kg actual body weight daily intake of total, animal, or plant-based protein in 2248 post-myocardial patients of the Alpha Omega Cohort.

		Total protein	Animal protein	Plant protein
Per 0.1 g/kg actual body weight	Crude	0.02 (-0.03; 0.07)	0.00 (-0.07; 0.07)	0.13 (0.00; 0.25)*
	Model 1	-0.12 (-0.20; -0.04)*	-0.12 (-0.20; -0.04)*	-0.06 (-0.24; 0.13)
	Model 2	-0.12 (-0.20; -0.04)*	-0.11 (-0.19; -0.03)*	-0.08 (-0.27; 0.11)
	Model 3	-0.12 (-0.21; -0.03)*	-0.12 (-0.21; -0.02)*	-0.14 (-0.37; 0.08)

*p<0.05
Model 1: adjusted for age, sex, body mass index, and total energy intake.
Model 2: Model 1 plus additional adjustment for education, alcohol, smoking, physical activity, RAS blocking drugs.
Model 3: Model 2 plus additional adjustment for intake of fat (mono- and poly-unsaturated fat, saturated fat, and trans fat), dietary sodium, diabetes, and systolic blood pressure; animal protein was also adjusted for plant protein, and vice versa.



Supplementary Figure S1: Flow chart of 2248 patients included in the present study. The patients randomized before August 2005 are considered a random sample of the total population of 4837 patients. Implausible high or low energy intake was defined as: <800 or >8000 kcal/day for men, <600 or >6000 kcal/day for women; n=7.

Chapter 5 –

Superior long-term survival for simultaneous pancreas-kidney transplantation as renal replacement therapy: 30-year follow-up of a nationwide cohort

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ABSTRACT

Objective: In patients with type 1 diabetes and end-stage renal disease, it is controversial whether a simultaneous pancreas-kidney (SPK) transplantation improves survival compared to kidney transplantation alone. We compared long-term survival in SPK and living or deceased donor kidney transplant recipients.

Research Design and Methods: We included all 2796 type 1 diabetes patients in The Netherlands, who started renal replacement therapy between 1986 and 2016. We used multivariable Cox regression analyses adjusted for recipient age and sex, dialysis modality and vintage, transplantation era, and donor age to compare all-cause mortality between deceased or living donor kidney and SPK transplant recipients. Separately, we analysed mortality between regions where SPK was the preferred intervention (80% SPK) *vs* regions where a kidney transplant alone was favoured (30% SPK).

Results: Of 996 transplanted patients, 42%, 16%, and 42% received a deceased or living donor kidney, or SPK transplant, respectively. Mean (SD) age at transplantation was 50 (11), 48 (11), and 42 (8) years, respectively. Median (95%-CI) survival time was 7.3 (6.2; 8.3), 10.5 (7.2; 13.7), and 16.5 (15.1; 17.9) years, respectively. SPK recipients with a functioning pancreas graft at one year (91%) had the highest survival (median 17.4 years). Compared to deceased donor kidney transplant recipients, adjusted hazard ratios (95%-CI) for 10- and 20-year all-cause mortality were 0.79 (0.49; 1.29) and 0.98 (0.69; 1.39) for living donor kidney, and 0.67 (0.46; 0.98) and 0.79 (0.60; 1.05) for SPK recipients, respectively. A treatment strategy favouring SPK over kidney transplantation alone showed 10- and 20-year mortality hazard ratios of 0.56 (0.40; 0.78) and 0.69 (0.52; 0.90), respectively.

Conclusions: Compared to living or deceased donor kidney transplantation, SPK was associated with improved patient survival, especially in recipients with a long-term functioning pancreatic graft, and resulted in an almost two-fold lower 10-year mortality rate.

INTRODUCTION

The global type 1 diabetes mellitus population approaches 40 million. Approximately 78,000 children are diagnosed with type 1 diabetes annually, and the incidence is expected to rise by 3% per year.[1] Micro- and macrovascular damage due to impaired glucose regulation leads to diabetic retinopathy, nephropathy, neuropathy, angiopathy and a three-fold increased mortality risk as compared to non-diabetic individuals.[2] As such, type 1 diabetes is accompanied by considerable health care costs, estimated at about 10,000 US dollars per patient per year.[3]

Patients with type 1 diabetes have a high cumulative risk of 7% to develop end-stage renal disease requiring renal replacement therapy within 30 years.[4] Compared with dialysis, kidney transplant recipients have a substantially improved survival and quality of life.[5, 6] In contrast to a kidney transplant alone, a simultaneous pancreas-kidney (SPK) transplantation may also restore endogenous insulin production and, at least partially, reverses progression of diabetic micro- and macrovascular complications.[7] Controversy remains however as to whether an SPK compared with a kidney transplant alone improves patient survival. Specifically, it is unknown whether an SPK should be preferred over a living donor kidney transplant.

For practical or ethical reasons, no randomised clinical trials have compared survival after SPK *vs* kidney transplantation alone. We previously showed, in Dutch type 1 diabetes patients between 1985 and 1996, that a treatment strategy favouring SPK over a deceased donor kidney transplant alone was associated with a 47% lower 10-year mortality risk.[8] In a US registry study among 18,549 type 1 diabetes patients during 1987-1996, eight-year survival after SPK or a living donor kidney transplant was similar at 72%, and better as compared to 55% in deceased donor kidney transplant recipients.[9] In the same registry during 2000-2007, recipients of a living donor kidney transplant had a better six-year survival as compared to SPK transplant patients, although others have found no clinically relevant 10-year survival benefit for SPK *vs* kidney transplantation alone.[10, 11] Weiss *et al* showed that SPK recipients who survived the first year post-transplant with a functioning pancreas graft, had a superior seven-year survival as compared to type 1 diabetes patients with a living donor kidney transplant (89% *vs* 80%).[12]

Taken together, there is no consensus on whether SPK compared with kidney transplantation alone actually improves mortality risk in patients with type 1 diabetes, especially in the long term. Therefore, we investigated the effect of SPK in comparison to kidney transplantation alone, either from a living or deceased donor, on long-term survival, in a nationwide cohort including all Dutch type 1 diabetes patients who have required renal replacement therapy in the past 30 years.

METHODS

Study population

We included consecutive (n=2833) type 1 diabetes mellitus patients aged at least 18 years, who started on chronic dialysis or received a first kidney transplant in the Netherlands between January 1, 1986 and January 1, 2016. We excluded patients who received a pancreas transplantation alone (n=17) or a pancreas after kidney transplantation (n=20); thus 2796 patients were eligible for the present analysis. In total, 1800 patients were on chronic dialysis only and 414, 161 and 421 patients received a deceased or living donor kidney, or SPK, respectively (Supplementary Figure S1). We used data from two mandatory nationwide Dutch registries. The Netherlands Organ Transplant Registry includes kidney transplant patients of all eight Dutch kidney transplant centres, containing information on donor and recipient characteristics as well as outcome parameters. The registry combines the donor, procurement and allocation data from the Eurotransplant Network Information System with transplant centre-specific data, and is updated annually. Registration of each organ transplantation is mandatory and is coordinated by the government via the Dutch Transplant Foundation. The Dutch Renal Registry (RENINE: Registratie Nierfunctievervanging Nederland) collects information on all chronic dialysis patients, registration for whom is also mandatory for all dialysis centres in order to receive funding. Data quality of both registries is periodically audited by on-site polls, application rules, and cross checks between the registries. Organs were allocated according to the standard Eurotransplant guidelines. Since type 1 diabetes patients on dialysis have a poor prognosis, Eurotransplant applies mandatory exchange rules for SPK, to prioritise this patient category in case of a potential SPK donor. These rules explain the shorter waiting time for SPK as compared to kidney transplantation alone, as well as the relatively large proportion of pre-emptive SPK transplant procedures (36%).[13] Deceased donor kidney and SPK transplants were performed following donation after brain death procedures in 95% of cases.

Regional differences in treatment strategy

The postal code of the type 1 diabetes patient strictly determines treatment in a defined dialysis centre, and each dialysis centre is affiliated to a specific transplant centre. Since the first pancreas transplant in the Netherlands in 1984, the Dutch Ministry of Health considered simultaneous pancreas-kidney transplantation an experimental and restricted procedure. The results has been that the vast majority of the simultaneous pancreas-kidney transplants have been performed in Leiden, which is only one of eight Dutch transplant

centres. These policies created regional differences in the assignment of simultaneous pancreas-kidney transplantation to patients with type 1 diabetes mellitus in essence largely based on their place of residence. We therefore defined two transplant areas: the Leiden area, with an average population of 2.5 million inhabitants during the 30-year follow-up period, and the rest of the Netherlands, with 14.0 million inhabitants. In the Leiden area, consisting of one transplantation centre, the primary intention is to treat type 1 diabetes patients with end-stage renal disease with an SPK. Thus, SPK was offered to the majority of type 1 diabetes patients. In contrast, in the non-Leiden area, consisting of seven transplantation centres, a kidney transplant alone has been the preferred treatment and SPK is performed in a significantly lower proportion of patients. Of all SPK transplants, 87% were performed in the Leiden area. Patients living in the Leiden area received an SPK in 80% of cases, compared with 30% for patients living in the non-Leiden area.

Importantly, immunosuppressive treatment for kidney transplant patients has changed over time. Until 1995 SPK recipients were treated with cyclosporine, azathioprine and prednisolone. From 1996 onward azathioprine was replaced by mycophenolate mofetil, and in 2003 cyclosporine was structurally replaced by tacrolimus. From 1997 induction therapy with intravenous anti-thymocyte globulin (ATG) was given, and beyond 2007 this was switched to subcutaneous alemtuzumab. For patients receiving a kidney transplant alone, immunosuppressive therapy changed comparably, although these patients do not receive ATG or alemtuzumab as induction therapy.

Endpoints

The primary endpoint was all-cause mortality. Patients were censored in case of loss to follow up, recovery of kidney function on dialysis, or end of follow-up (January 1, 2016), whichever came first. We defined patient survival as the time between start of dialysis or first kidney transplantation with or without pancreas transplant and the date of death from any cause. Pancreatic graft failure was defined as pancreas graft loss, need for exogenous insulin, or serum C-peptide levels <0.3 nmol/L. The secondary outcome was kidney graft failure, defined as kidney graft loss after transplantation and return to dialysis. We defined graft survival as the time between the date of transplantation and the date of graft failure or death. We investigated both graft failure including all-cause mortality, and death-censored graft failure. Finally, we assessed the occurrence of delayed graft function, defined as the need for dialysis within the first week after surgery, for the three different types of transplantation (deceased or living donor kidney, and SPK). Kidney grafts that never functioned were not considered as delayed graft functioning.

Statistical analyses

Baseline recipient and donor characteristics are presented as mean (SD) or number (%), when appropriate; data are presented for all patients, for different types of renal replacement therapy, and for different regions. There were no missing data for the most important clinical parameters; nine patients (0.3%) were lost to follow-up.

First, survival was compared between different types of transplantation. Crude survival was presented by Kaplan-Meier curves. Adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) for 10- and 20-year all-cause mortality were estimated by Cox regression. Analyses were adjusted for recipient age and sex, donor age, dialysis vintage and modality, and year of transplantation (per five-year interval). We adjusted for year of transplantation to account for changes in treatment protocols and medical care. To visualise the cumulative incidence of kidney graft failure, taking into account death as a competing risk, we used competing risk regression according to Fine and Gray.[14] Adjusted cause-specific HRs for kidney graft failure were calculated using standard Cox regression analyses, censoring patients in case of death.[15] Additionally, we investigated the influence of changes in immunosuppressive therapy over time on survival of SPK recipients. We therefore chose to compare 10-year all-cause mortality of SPK recipients transplanted in the period 1986-1999 and 2000-2015. We also investigated the influence of a long-term (defined as at least one year) functioning pancreas graft in SPK recipients on mortality. Information on date of pancreatic graft failure was only available for patients transplanted in the Leiden area (367 patients, 87% of all SPK recipients). We included all transplanted patients alive one year after transplantation, and stratified SPK recipients on having a functioning or failed pancreas graft.

Second, we performed analyses at the regional level (Leiden *vs* non-Leiden), to mimic an "intention-to-treat" analysis.[8] We provide effect estimates of SPK *vs* kidney transplant alone, by analysing patients according to their region of residence, and not according to the region where they were actually transplanted. Under the assumption that medical care for transplant patients is similar in the Leiden and non-Leiden areas, and that prognostic factors are similar for patients in both areas, confounding is dealt with by design. For example, a patient living in the non-Leiden area, but who received an SPK transplant in Leiden, was analysed according to the intended treatment belonging to the non-Leiden area.[8] Patients living in the Leiden and non-Leiden areas received an SPK transplant in 80% and 30% cases, respectively. Overall survival of transplanted patients was compared between the Leiden and non-Leiden areas. HRs for 10- and 20-year all-cause mortality were calculated using Cox regression, adjusted for recipient age and sex, donor age, dialysis vintage

and modality, and year of transplantation (per five-year interval). We compared survival on dialysis for the Leiden *vs* non-Leiden areas, censoring patients when transplanted.

Finally, survival was compared in patients who received any form of kidney transplantation (deceased or living donor kidney, or SPK) versus chronic dialysis treatment. In these analyses only dialysis patients on the waiting list for transplantation were included, to increase comparability of clinical characteristics between dialysis and transplanted patients. Dialysis and transplantation patients were matched for dialysis vintage, to avoid immortal time bias and minimise confounding by dialysis vintage. Survival time in transplanted patients was counted from the date of transplantation, and for matched dialysis patients we subtracted the dialysis vintage of the transplanted match, thereby creating a similar start of follow-up. Differences in crude survival were tested by the Log-rank test. HRs for five- and 10-year all-cause mortality were calculated using Cox regression, adjusted for recipient age and sex, and year of renal replacement therapy initiation (per five-year interval).

In all Cox regression analyses, the proportional hazards assumption was not violated, demonstrated by parallel log-survival curves in log-minus-log plots.[16] We repeated all analyses in patients who survived the first three months without graft loss. We thus excluded surgically- and immunologically-related death. We considered two-sided *p*-values <0.05 statistically significant. All analyses were performed using STATA Statistical Software version 14 (Statacorp, Texas, USA) and SPSS 25.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Baseline characteristics

Of all 2796 type 1 diabetes patients, 996 (36%) received a first kidney transplant from either a deceased (42%) or living (16%) donor, and 42% received an SPK (Table 1). Approximately 35% and 42% of living donor kidney and SPK recipients were pre-emptively transplanted. Mean (SD) age at start of dialysis was 59 years (13) for patients who stayed on chronic maintenance dialysis, and was 44 years (10) for transplant recipients. For SPK, both recipient age at transplantation and donor age were younger as compared to deceased or living donor kidney transplant recipients. Recipients of a deceased donor kidney had the longest dialysis vintage before transplantation and a longer cold ischemic period as compared to recipients of a living donor kidney or SPK. Delayed graft function occurred in 122 (12%) of all transplanted patients. For deceased donor kidney recipients the incidence of delayed graft failure was 25%, compared to 6%

and 2% for recipients of a living donor kidney or SPK transplant. Patients from the Leiden *vs* non-Leiden area had comparable age and sex distribution (Supplementary Table S1).

Table 1: Baseline characteristics 2796 type 1 diabetes mellitus patients, according to type of renal replacement therapy.

	Dialysis n=1800	**DDKT** n=414	**LDKT** n=161	**SPKT** n=421
Age at dialysis, y	59 ± 13	47 ± 10	46 ± 11	40 ± 8
Age at transplantation, y	-	50 ± 11	48 ± 11	42 ± 8
Men, %	53	63	58	62
Donor age, y	-	42 ± 16	51 ± 12	34 ± 12
Dialysis modality, %				
Hemodialysis	71	37	35	26
Peritoneal dialysis	29	34	23	31
Missing	0.1	14	7	1
Pre-emptive Tx, %	-	15	35	42
Dialysis vintage, mo [a]	36 ± 34	26 ± 24	12 ± 18	12 ± 19
Cold ischaemic time, h	-	23 ± 9	2 ± 1	13 ± 4
Place of residence, %				
Leiden area	14	8	9	45
Non-Leiden area	86	92	91	55

[a] Excluding pre-emptive transplant patients
Numbers are presented as mean ± SD or percentage.
DDKT, deceased donor kidney transplant; LDKT, living donor kidney transplant; SPKT, simultaneous pancreas kidney transplantation; Tx, transplantation.

Simultaneous pancreas-kidney transplantation compared to kidney transplantation alone

Crude survival was highest in SPK recipients, and lowest in recipients of a deceased donor kidney (Figure 1A). Compared to the latter patient group, adjusted HRs (95%-CI) for 10-year all-cause mortality for living donor kidney and SPK recipients were 0.79 (0.49; 1.29) and 0.67 (0.46; 0.98), and for 20-year all-cause mortality were 0.98 (0.69; 1.39) and 0.79 (0.60; 1.05), respectively (Table 2). The HR (95%-CI) for 10-year and 20-year all-cause mortality for SPK compared to living donor kidney recipients was 0.85 (0.53; 1.38) and 0.81 (0.57; 1.16), respectively. Overall graft loss, defined as death or kidney graft failure,

was dominated by patient mortality, and therefore results were comparable to those for all-cause mortality alone. Recipients of a living donor kidney had the lowest cumulative incidence of death-censored kidney graft failure, while death-censored graft failure was comparable for deceased donor kidney and SPK recipients (Figure 1B). Compared with deceased donor kidney recipients, the adjusted HR (95%-CI) for 10-year death-censored kidney graft failure was 0.52 (0.28; 0.98) and 1.05 (0.66; 1.67) for living donor kidney and SPK recipients, respectively (Table 2). Repeating analyses restricted to type 1 diabetes patients who survived the first three months after initiation of dialysis or kidney transplantation, yielded similar results.

Table 2: Hazard ratios (95%-CIs) for 10-year and 20-year all-cause mortality and death-censored kidney graft failure for living kidney transplantation or deceased kidney transplantation with or without simultaneous pancreas transplantation.

	Crude	Model 1	Model 2	Model 3
10-year all-cause mortality				
DDKT (ref)	1	1	1	1
LDKT	0.57 (0.37; 0.86)	0.64 (0.42; 0.98)	0.56 (0.36; 0.86)	0.79 (0.49; 1.29)
SPKT	0.34 (0.25; 0.45)	0.41 (0.30; 0.56)	0.44 (0.32; 0.61)	0.67 (0.46; 0.98)
10-year death-censored graft failure				
DDKT (ref)	1	1	1	1
LDKT	0.61 (0.35; 1.06)	0.59 (0.34; 1.02)	0.38 (0.21; 0.67)	0.52 (0.28; 0.98)
SPKT	0.67 (0.46; 0.97)	0.60 (0.41; 0.89)	0.76 (0.50; 1.15)	1.05 (0.66; 1.67)
20-year all-cause mortality				
DDKT (ref)	1	1	1	1
LDKT	0.69 (0.51; 0.94)	0.75 (0.55; 1.03)	0.70 (0.50; 0.96)	0.98 (0.69; 1.39)
SPKT	0.44 (0.36; 0.56)	0.55 (0.44; 0.71)	0.58 (0.45; 0.74)	0.79 (0.60; 1.05)
20-year death-censored graft failure				
DDKT (ref)	1	1	1	1
LDKT	0.63 (0.38; 1.03)	0.60 (0.37; 0.98)	0.40 (0.24; 0.67)	0.50 (0.29; 0.88)
SPKT	0.59 (0.42; 0.83)	0.52 (0.37; 0.74)	0.62 (0.43; 0.89)	0.79 (0.53; 1.20)

DDKT, deceased donor kidney transplant; LDKT, living donor kidney transplant; SPKT, simultaneous pancreas kidney transplantation.
Model 1: Adjusted for recipient age and sex.
Model 2: Model 1, plus adjustment for donor age.
Model 3: Model 2, plus adjustment for dialysis vintage, dialysis modality, and transplantation era.

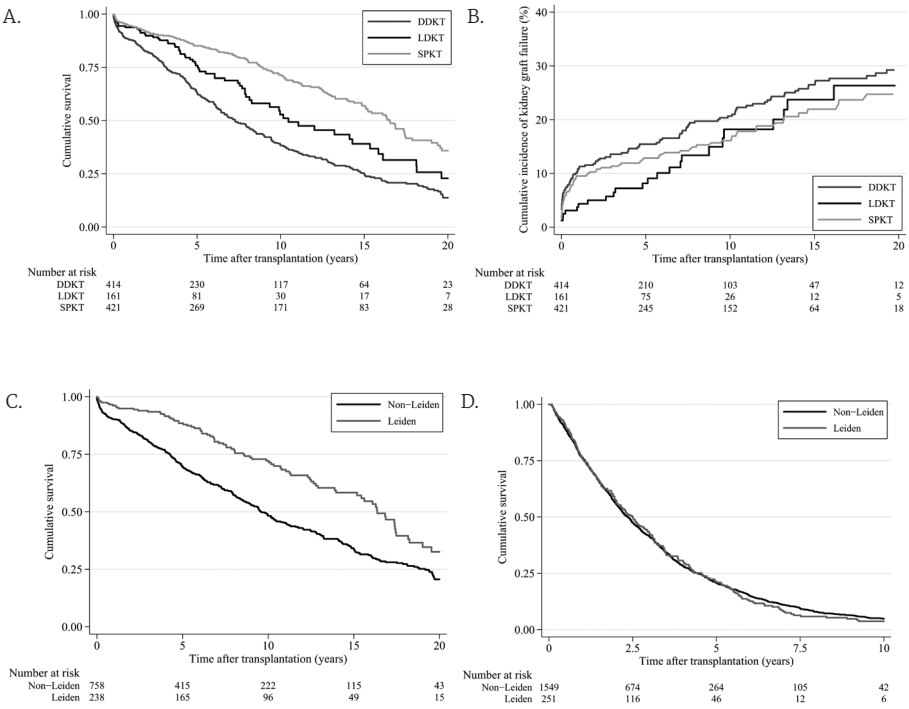

Figure 1. Crude Survival curves. A: Overall survival of patients with type 1 diabetes after DDKT, LDKT, or SPKT. Median (95% CI) survival time was 7.3 (6.2; 8.3) years for patients with DDKT, 10.5 (7.2; 13.7) years for patients with LDKT, and 16.5 (15.1; 17.9) years for patients with SPKT. **B:** Cumulative incidence of kidney graft failure, taking into account the competing risk of death. **C:** Survival of patients with type 1 diabetes after transplantation in the Leiden area vs. the non-Leiden area. Median (95% CI) survival was 9.6 (8.6; 10.6) years for the non-Leiden area and 16.4 (14.9; 17.8) years for the Leiden area. **D:** Survival of patients with type 1 diabetes during dialysis in the Leiden area vs. the non-Leiden area. Median (95%CI) survival was 3.1 (3.0; 3.3) years for the non-Leiden area and 3.2 (2.8; 3.5) years for the Leiden area. DDKT, deceased-donor kidney transplant; LDKT, living-donor kidney transplant; SPKT, SPK transplantation.

In total, 137 and 284 SPK transplantations were performed between 1986-1999 and 2000-2015, respectively, with mean (SD) recipient age 39 (7) years and 43 (8) years, and donor age 30 (11) years and 35 (12) years, respectively. Kaplan-Meier estimates for 10-year survival for SPK recipients transplanted between 2000-2015 was 77%, and 63% for those transplanted between 1986-1999 (Supplementary Figure S2). The HR (95%-CI) for 10-year mortality was 0.48 (0.30; 0.76) for SPK recipients transplanted between 2000-2015, as compared to the period 1986-1999 (Supplementary Table S2). Comparable but slightly attenuated HRs were observed for deceased and living donor transplant recipients (Supplementary Table S2).

Of all 367 SPK recipients transplanted in the Leiden area who survived the first postoperative year, 34 experienced pancreas graft failure. Patients with a functioning pancreas graft at one year had a 10-year survival of 80%, while patients who experienced pancreas graft failure showed survival comparable to recipients of a deceased donor kidney transplant, being less than 50% (Supplementary Figure S3). Median (95%-CI) survival for SPK recipients with a functioning pancreas graft, or recipients of a living or deceased donor kidney was 17.4 (15.4; 19.5), 12.0 (8.0; 16.0), and 8.6 (7.4; 9.7) years, respectively. SPK recipients with pancreas graft failure had a 2.15 (95%-CI: 1.09; 4.27) and 1.42 (95%-CI: 0.77; 2.62) times higher 10-year and 20-year all-cause mortality risk than those with a functioning pancreas at one year (Table 3). In patients who survived the first postoperative year, SPK recipients who experienced pancreas graft failure had a comparable survival to recipients of a deceased donor kidney transplant alone (Table 3).

Table 3: Hazard ratios (95%CI) of 10-year and 20-year all-cause mortality for different types of kidney transplantation with or without simultaneous pancreas transplantation, conditional on surviving the first year after transplantation.

	Crude	Model 1	Model 2	Model 3
10-year all-cause mortality				
DDKT (ref)	1	1	1	1
LDKT	0.67 (0.46; 0.99)	0.72 (0.49; 1.07)	0.59 (0.39; 0.88)	0.74 (0.48; 1.15)
SPKT panc (+)	0.26 (0.18; 0.38)	0.32 (0.22; 0.47)	0.35 (0.24; 0.52)	0.44 (0.29; 0.68)
SPKT panc (–)	0.82 (0.46; 1.44)	0.99 (0.55; 1.79)	1.01 (0.56; 1.83)	1.10 (0.60; 2.05)
SPKT panc (+) (ref)	1	1	1	1
SPKT panc (–)	3.15 (1.67; 5.93)	2.91 (1.50; 5.63)	2.60 (1.34; 5.05)	2.15 (1.09; 4.27)
20-year all-cause mortality				
DDKT (ref)	1	1	1	1
LDKT	0.76 (0.54; 1.06)	0.82 (0.59; 1.15)	0.72 (0.51; 1.03)	0.94 (0.65; 1.37)
SPKT panc (+)	0.38 (0.28; 0.50)	0.45 (0.33; 0.61)	0.48 (0.35; 0.65)	0.62 (0.45; 0.87)
SPKT panc (–)	0.73 (0.43; 1.24)	0.88 (0.51; 1.50)	0.88 (0.51; 1.51)	1.04 (0.59; 1.83)
SPKT panc (+) (ref)	1	1	1	1
SPKT panc (–)	1.99 (1.14; 3.47)	1.83 (1.01; 3.30)	1.64 (0.90; 2.97)	1.42 (0.77; 2.62)

DDKT, deceased donor kidney transplant; LDKT, living donor kidney transplant; SPKT, simultaneous pancreas kidney transplantation; panc (+), with functioning pancreatic graft after 1 year; panc (–), with pancreatic graft failure within one year.
Model 1: Adjusted for recipient age and sex.
Model 2: Model 1, plus adjustment for donor age.
Model 3: Model 2, plus adjustment for dialysis vintage, dialysis modality, and transplantation era.

Regional differences in intended treatment

In total, 238 patients were transplanted in the Leiden and 758 patients in the non-Leiden area (Supplementary Table S1). Survival for transplanted type 1 diabetes patients was higher in the Leiden compared to non-Leiden area (Figure 1C). Median (95%-CI) survival time was 16.4 (14.9; 17.8) and 9.6 (8.6; 10.6) years for the patients residing in the Leiden *vs* non-Leiden area. After multivariable adjustment, the HR (95%-CI) for 10-year and 20-year all-cause mortality for Leiden *vs* non-Leiden was 0.56 (0.40; 0.78) and 0.69 (0.52; 0.90), respectively (Supplementary Table S3), and quite similar to unadjusted estimates. Exclusion of pre-emptively transplanted patients yielded comparable results, with a HR for 10-year all-cause mortality of 0.52 (0.34; 0.80). We found no significant difference with regard to death-censored graft failure: 10-year cause-specific HR 0.88 (95%-CI: 0.55; 1.39) for patients living in the Leiden *vs* non-Leiden area. Survival on chronic dialysis was similar in both regions (Figure 1D), reflected by an adjusted HR for five-year mortality of 0.97 (95%-CI: 0.83; 1.13).

Dialysis compared to kidney transplantation

Compared to patients on the waiting list, dialysis patients not on the waiting list for transplantation, , had a 1.54 (95%-CI: 1.34; 1.78) times higher five-year mortality risk (Supplementary Table S4). Survival was better for transplanted patients compared with chronic dialysis patients on the waiting list (Supplementary Figure S4). Five-year survival was 32% for wait-listed dialysis patients *vs* 76% for transplanted patients. The adjusted HR for five-year all-cause mortality was 0.25 (0.19; 0.32) for transplanted patients, compared with dialysis patients on the waiting list (Supplementary Table S4). HRs for 10-year mortality were comparable.

DISCUSSION

In this Dutch nationwide cohort including all type 1 diabetes patients who started renal replacement therapy between 1986 and 2016, those who received an SPK had a 20-30% lower 10- and 20-year all-cause mortality risk compared to recipients of a deceased donor kidney transplant. The risk of 20-year all-cause mortality for SPK compared with living donor kidney recipients was 20% lower, despite the fact that living donor kidney recipients had better kidney graft survival. Patient survival was highest for SPK recipients with a functioning pancreas graft at one year. In contrast, survival for SPK recipients who lost their pancreas graft within one year was comparable to recipients of a deceased donor kidney transplant alone. Most importantly, a treatment

strategy with the primary intention of treating patients with an SPK resulted in an almost 50% reduction in 10-year all-cause mortality risk compared to a kidney transplant alone.

We performed the present analyses to aid in the ongoing controversy whether a SPK transplant as compared to a kidney transplant alone lowers mortality risk in patients with type 1 diabetes and end-stage renal failure, especially on the long term. This is the first study that clearly shows that type 1 diabetes patients, both 10 and 20 years after simultaneous pancreas-kidney transplant, had a substantially higher life expectancy, as compared to those who received a living or deceased donor kidney transplant alone.[17, 18] Most previous studies have followed patients for less than 10 years providing conflicting results.[9-12] Moreover, post-transplant healthcare rapidly improved in the past decades, while most previous studies reported data up to 2010. We followed patients up to 2016 and separately report the results obtained before and after 2000. For example, the wide introduction of the different forms of induction therapy markedly improved outcomes for both kidney and simultaneous pancreas-kidney transplantation. Alemtuzumab, for instance, is since 2007 part of our SPK protocol and resulted in the most pronounced improvement in outcome parameters.[19]

The HR (95%-CI) for 10- and 20-year all-cause mortality for SPK *vs* living donor kidney transplant recipients was 0.85 (0.53; 1.38) and 0.81 (0.57; 1.16). Importantly, living donor kidney transplant recipients less often experienced death-censored kidney graft failure. This implies that the improved survival after SPK transplantation may be explained by the eliminated need for exogenous insulin and reduction of non-renal diabetic complications. Indeed, we showed that median survival of SPK recipients with a functioning pancreas graft one-year after transplantation was 17.4 *vs* 10.7 years for those with pancreas graft failure. Median survival was 8.6 years for deceased and 12.0 years for living donor kidney recipients. These results confirm previous data by Weiss *et al*.[12] In contrast to the present study, Ojo *et al* observed comparable 10-year crude survival rates for SPK and living donor kidney transplant recipients of 67% and 65%, respectively.[18] Comparable survival rates were found by others.[9, 20-23] Sung *et al* concluded that, up to 10 years, SPK transplantation as compared to kidney transplantation alone was associated with a clinically irrelevant survival benefit of 0.17 years. Using the same data registry, a subsequent analysis found that with a follow-up extended beyond 10 years, the survival benefit for SPK increased as compared to kidney transplant alone.[11, 24] Previous studies investigated patient cohorts with, at most, 10 years of follow-up.

The overall five-year survival of SPK recipients in general improved from 75% to 90% between 1990-2009.[25] Differences in treatment regimens, especially introduction of T-cell depleting agents such as induction therapy, have drastically reduced the incidence of acute rejection episodes in SPK

recipients.[26, 27] Until 1997, no induction therapy was given, leading to over 80% acute rejections after SPK transplantation. Ringers *et al* showed that ATG induction or interleukin-2 receptor blockade reduced the rate of acute rejection to about 40%.[28] Induction with alemtuzumab instead of ATG from 2007 onwards further reduced the incidence of acute rejection.[19] A therapy regimen including tacrolimus instead of cyclosporine was introduced in 2003, and resulted in fewer and less severe kidney and pancreas rejections.[29] The more recent sample of patients included in the present study is more generalisable to current clinical practice. Indeed, we showed that 10-year mortality risk was about halved for type 1 diabetes patients who received an SPK between 2000-2015, as compared to those transplanted in the period 1986-1999, despite increased mean donor and recipient ages during the latter period.

Using regional differences in treatment strategies, we showed that the approach favouring SPK had superior 10- and 20-year survival as compared to one advocating kidney transplantation alone. Since we did not expect origin-related variables, we used these regional differences to mimic an intention-to-treat approach, reducing the influence of confounders such as age and dialysis vintage. On average, recipients and donors for SPK were younger than those for a living or deceased donor kidney transplant. We showed that our intention-to-treat approach resulted in more similar patient groups as opposed to comparing transplant by type, which is also reflected by the similar mortality rates for patients on dialysis in both regions. Importantly, we showed that survival while on dialysis was almost identical between the two regions (HR 0.97), suggesting that differences in care are unlikely to explain our results. These results imply that SPK compared to kidney transplantation alone led to improved patient survival, which is in line with an earlier comparable Dutch study analysing patients until 1996.[8]

The main advantage of a pancreas transplantation in addition to a kidney transplantation is the improved quality of life due to resolving the need for exogenous insulin.[5, 7] Furthermore, curing diabetes halts an otherwise ongoing progression of diabetic complications, in particular nephropathy, retinopathy, and neuropathy.[30-32] Finally, pancreas transplantation was shown to attenuate progression of atherosclerosis and improve cardiac functioning.[33, 34] In contrast, short-term mortality may be higher for SPK as compared to kidney transplantation alone, owing to the more complicated nature of the procedure. However, most studies assessing short-term survival for transplanted type 1 diabetes patients reported comparable short-term survival for SPK and living donor kidney recipients.[35]

The survival benefit of a kidney transplant as compared to remaining on dialysis is well known.[36] Others have shown that adjusted hazard ratios for

5-year mortality, using wait-listed dialysis patients as reference, were 0.40, 0.45, and 0.75 for SPK, living, and deceased kidney transplants, respectively.[18] Transplanted type 1 diabetes patients compared to those on the waiting list while on dialysis had a four-fold reduction in five-year mortality risk.

This study has several limitations. First, data collection in a registry study may have led to misclassification, measurement error, and missing data. However, in the present study the proportion of missing data of key variables was negligible, and regular quality cross-checks between the two mandatory registries reduced the risk of misclassification. Additionally, inherent to using registry data, we had limited information about important patient characteristics, such as lifestyle, comorbidity, and medical history. Second, we compared several interventions in an observational study. Despite adjusting for confounders, residual confounding may remain. We aimed to limit the influence of confounding by also using regional differences to compare intended treatment strategies. Because our main analysis was based on a comparison of two treatment strategies (preferably SPK *vs* preferably non-SPK), our study did not clarify which patients actually benefited most from an SPK transplant. Third, we had no detailed data on the cardiovascular risk profile of the type 1 diabetes patients eligible for kidney transplantation. However, all type 1 diabetes patients in The Netherlands with renal insufficiency are managed according to the latest KDIGO guidelines.[37] In addition, the approval for kidney or SPK occurs in each transplantation centre according to a nationwide consensus based on international guidelines.[38]

The main strength of the present study is the nationwide sample, including all type 1 diabetes patients in The Netherlands requiring renal replacement therapy during a 30-year period. Furthermore, we used regional differences to mimic an intention-to-treat principle, reducing the influence of confounding.

In conclusion, in type 1 diabetes patients with end-stage renal disease, a treatment strategy favouring SPK compared to kidney transplantation alone, was associated with a 44% and 31% reduction of 10- and 20-year all-cause mortality, respectively. SPK recipients with a functioning pancreas graft had an approximately 50% reduced mortality risk as compared to those with a failed pancreas graft in the first year, and also experienced better survival in comparison to living donor kidney transplant recipients. These results encourage care providers and guidelines to adopt SPK transplantation as the preferred treatment option for type 1 diabetes patients with or approaching end-stage renal disease.

DISCLOSURES

We declare no conflicts of interest.

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This study did not receive external funding.

AUTHORS' CONTRIBUTIONS

Research idea and study design: JF, OD, PB, KE; data acquisition: KE, MM, PB, CK, Dutch Transplantation Foundation; data analysis/interpretation: JF, EH, OD, KE; statistical analysis: KE, OD; supervision and mentorship: JF, EH, OD. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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SUPPLEMENTARY DATA

Supplementary Table S1: Baseline characteristics 2796 type 1 diabetes patients, according to area of residence and type of renal replacement therapy.

	Dialysis		Transplantation	
	Leiden n=251	Non-Leiden n=1549	Leiden n=238	Non-Leiden n=758
Age at dialysis, y	58 ± 13	59 ± 13	43 ± 10	44 ± 10
Age at transplantation, y			44 ±10	46 ± 11
Men, %	53	53	59	63
Donor age, y	-	-	36 ± 14	41 ± 16
Dialysis modality, nr (%)				
Haemodialysis	68	72	22	35
Peritoneal dialysis	32	28	21	34
Missing	0	0.1	0	10
Pre-emptive Tx, %			57	21
Dialysis vintage, mo	9 ± 6	8 ± 6	23 ± 24	20 ± 22
Cold ischaemic time, h	-	-	15 ± 8	16 ± 11
DDKT, nr (%)	-	-	14	50
LDKT, nr (%)	-	-	6	20
SPKT, nr (%)	-	-	80	30

[a] Excluding pre-emptive transplant patients
Numbers are presented as mean ± SD or percentage.
DDKT, deceased donor kidney transplant; LDKT, living donor kidney transplant; SPKT, simultaneous pancreas kidney transplantation; Tx, transplantation.

Supplementary Table S2: Hazard ratios of 10-year mortality of patients transplanted until the year 2000, compared with patients transplanted afterwards.

	Crude	Model 1	Model 2	Model 3
DDKT				
1986 to 1999 (ref)	1	1	1	1
2000 to 2015	0.74 (0.54; 1.03)	0.62 (0.44; 0.87)	0.57 (0.40; 0.81)	0.54 (0.37; 0.78)
LDKT				
1986 to 1999 (ref)	1	1	1	1
2000 to 2015	0.95 (0.47; 1.90)	0.58 (0.27; 1.24)	0.57 (0.27; 1.21)	0.56 (0.26; 1.19)
SPKT				
1986 to 1999 (ref)	1	1	1	1
2000 to 2015	0.60 (0.39; 0.94)	0.51 (0.32; 0.81)	0.48 (0.30; 0.76)	0.48 (0.30; 0.76)

Model 1: Adjusted for recipient age and sex.
Model 2: Model 1, plus adjustment for donor age.
Model 3: Model 2, plus adjustment for dialysis vintage and dialysis modality.

Supplementary Table S3: Hazard ratios (95%CI) of 10- and 20-year all-cause mortality and death-censored graft failure for kidney transplanted patients living in the Leiden area compared to the non-Leiden area.

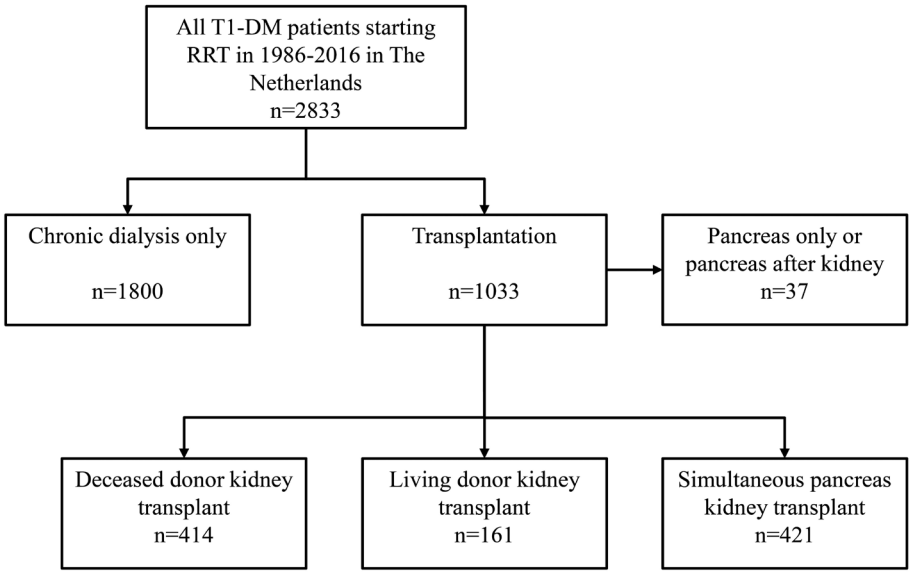
	Crude	Model 1	Model 2	Model 3
10-year all-cause mortality				
Non-Leiden area (ref)	1	1	1	1
Leiden area	0.47 (0.34; 0.64)	0.50 (0.37; 0.69)	0.54 (0.39; 0.73)	0.56 (0.40; 0.78)
10-year death-censored graft failure				
Non-Leiden area (ref)	1	1	1	1
Leiden area	0.74 (0.49; 1.11)	0.73 (0.48; 1.09)	0.87 (0.57; 1.33)	0.88 (0.55; 1.39)
20-year all-cause mortality				
Non-Leiden area (ref)	1	1	1	1
Leiden area	0.56 (0.44; 0.72)	0.60 (0.47; 0.78)	0.63 (0.48; 0.81)	0.69 (0.52; 0.90)
20-year death-censored graft failure				
Non-Leiden area (ref)	1	1	1	1
Leiden area	0.69 (0.48; 1.00)	0.68 (0.47; 0.99)	0.80 (0.55; 1.16)	0.79 (0.52; 1.19)

Model 1: Adjusted for recipient age and sex.
Model 2: Model 1, plus adjustment for donor age.
Model 3: Model 2, plus adjustment for dialysis vintage, dialysis modality, and transplantation era.

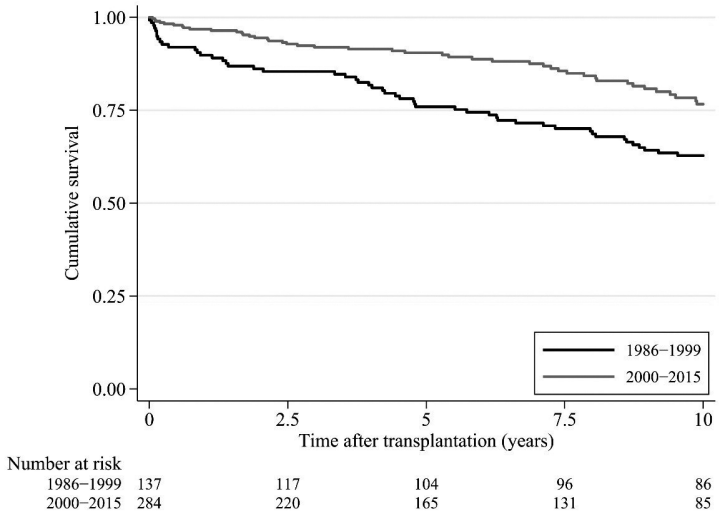
Supplementary Table S4.: Hazard ratios (95%CI) of 5-year and 10-year mortality for type 1 diabetes after kidney transplantation compared with dialysis, matched for dialysis vintage.

	Crude	Model 1	Model 2
5-year mortality			
Dialysis (on waiting list)	1	1	1
Dialysis (not on waiting list)	1.70 (1.50; 1.93)	1.59 (1.38; 1.83)	1.54 (1.34; 1.78)
Transplantation*	0.23 (0.18; 0.30)	0.24 (0.18; 0.31)	0.25 (0.19; 0.32)
10-year mortality			
Dialysis (on waiting list)	1	1	1
Dialysis (not on waiting list)	1.62 (1.44; 1.81)	1.50 (1.32; 1.70)	1.46 (1.29; 1.66)
Transplantation*	0.21 (0.17; 0.26)	0.22 (0.18; 0.28)	0.23 (0.18; 0.28)

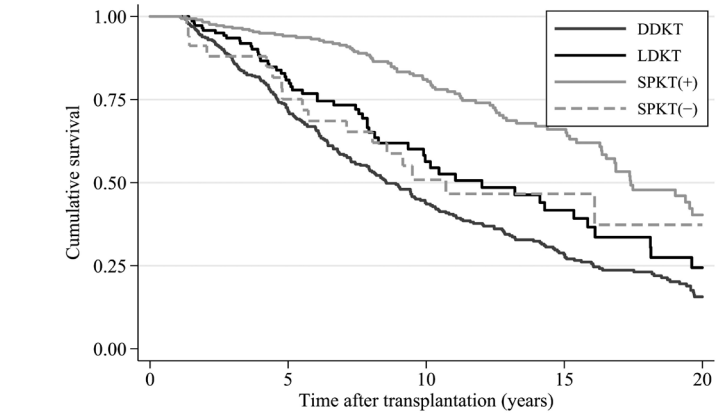
*Transplantation included both living kidney donor transplant, or deceased donor transplant with or without simultaneous pancreas transplantation.
Model 1: Adjusted for age and sex.
Model 2: Model 1, plus adjustment for calendar time.



Supplementary Figure S1: Flow diagram of 2833 type 1 diabetes mellitus (TI-DM) patients with end-stage renal disease, and different types of renal replacement therapy (RRT).



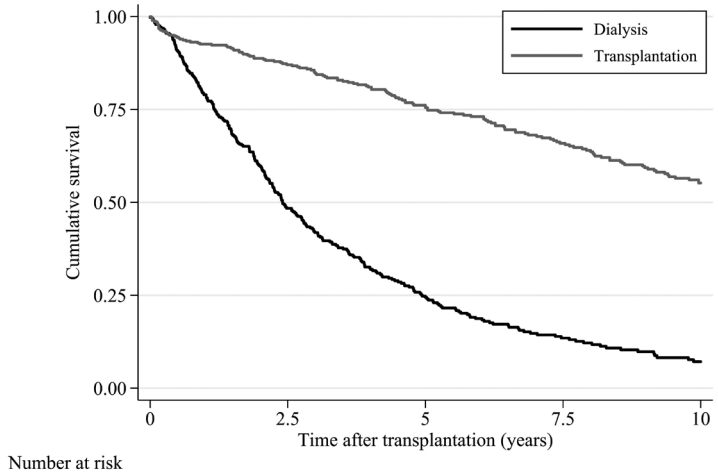
Supplementary Figure S2: Survival of simultaneous pancreas kidney transplantation (SPKT) patients transplanted in the period 1986-1999 and 2000-2015.



Number at risk

DDKT	361	230	117	64	23
LDKT	150	81	30	17	7
SPKT(+)	305	217	137	67	20
SPKT(−)	34	23	12	5	4

Supplementary Figure S3: Type 1 diabetes mellitus patient survival conditional on survival of the first year after transplantation, according to transplantation type: living donor kidney transplant (LDKT), deceased donor kidney transplant (DDKT), and simultaneous pancreas kidney transplantation (SPKT). SPKT patients were divided into patients with a functioning pancreatic graft after one year, SPKT(+), and those with pancreatic graft failure in the first year, SPKT(-). Median survival was 8.6 (7.4; 9.7) years for DDKT, 12.0 (8.0; 16.0) years for LDKT, 17.4 (15.4; 19.5) years for SPKT(+), and 10.7 (3.5; 17.9) years for SPKT(-). DDKT, deceased donor kidney transplant; LDKT, living donor kidney transplant; SPKT, simultaneous pancreas kidney transplantation.



Number at risk

Dialysis	379	157	70	31	12
Transplantation	379	301	226	177	131

Supplementary Figure S4: Patient survival after start dialysis or kidney transplantation. Each transplanted patient was matched on dialysis vintage with a chronic dialysis patient on the waiting list for transplantation. Median survival was 2.4 (2.1; 2.7) years for dialysis patients, and 11.3 (9.6; 12.9) years for transplanted patients.

Chapter 6 –

Effect of different types of statins on kidney function decline and proteinuria: a network meta-analysis

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ABSTRACT

Background: Previous studies showed that statins reduce the progression of kidney function decline and proteinuria, but whether specific types of statins are more beneficial than others remains unclear. We performed a network meta-analysis of randomized controlled trials (RCT) to investigate which statin most effectively reduces kidney function decline and proteinuria.

Methods: We searched MEDLINE, Embase, Web of Science, and the Cochrane database until July 13, 2018, and included 43 RCTs (>110,000 patients). We performed a pairwise random-effects meta-analysis and a network meta-analysis according to a frequentist approach. We assessed network inconsistency, publication bias, and estimated for each statin the probability of being the best treatment.

Results: Considerable heterogeneity was present among the included studies. In pairwise meta-analyses, 1-year use of statins versus control reduced kidney function decline by 0.61 (95%-CI: 0.27; 0.95) mL/min/1.73m^2 and proteinuria with a standardized mean difference of -0.58 (-0.88; -0.29). The network meta-analysis for the separate endpoints showed broad confidence intervals due to the small number available RCTs for each individual comparison.

Conclusions: 1-year statin use versus control attenuated the progression of kidney function decline and proteinuria. Due to the imprecision of individual comparisons, results were inconclusive as to which statin performs best with regard to renal outcome.

INTRODUCTION

Chronic kidney disease (CKD) is an increasing global health burden owing to population ageing and unhealthier lifestyle.[1] Up to 11% of the European population aged 45y or older has CKD stage 3, defined as an estimated glomerular filtration rate (eGFR) below 60 mL/min/1.73m^2.[2] CKD is an independent risk factor for cardiovascular morbidity and mortality.[3] Nowadays, the most important causes of CKD are cardiovascular disease, hypertension, diabetes, smoking, and hypercholesterolemia.[4,5] Generally, patients with symptomatic cardiovascular disease are prescribed cholesterol-lowering medication for secondary cardiovascular prevention. The latest KDIGO guideline on lipid management in CKD, recommends treatment with a statin in all non-dialysis dependent CKD patients ≥50 years with an eGFR below 60 mL/min/1.73m^2 or with at least 30 mg/g albuminuria, independent of serum cholesterol levels, which is also stated by the 2016 ESC/EAS guidelines.[6,7] Younger patients should use a statin in case of elevated cardiovascular risk, such as diabetes or coronary heart disease. Finally, statins should be continued, but not initiated, in patients on dialysis.[6] Multiple meta-analyses studied the effect of statins on renal outcomes. Recently, a meta-analysis by Su *et al.* concluded that statin users *vs* nonusers have a slower rate of kidney function decline and less proteinuria.[8]

Targeted prevention of kidney function decline is important to improve life expectancy and quality of life. However, it remains unclear whether specific types of statins are more beneficial than others regarding slowing down kidney function decline and lowering proteinuria. Various statins have different characteristics in terms of half-life, structure, lipophilicity, and potency.[9] We therefore performed a network meta-analysis of randomized controlled trials in adults that compare any statin with another statin or control treatment, to investigate which statin most effectively reduces kidney function decline or proteinuria. Network meta-analyses take into account both direct and indirect evidence of multiple comparisons in a treatment network, and provide information on which treatment performs best. These results may inform future guidelines about prevention of CKD and slowing down its progression.

METHODS

Systematic literature review

We performed a systematic review of the literature, searching MEDLINE, Embase, Web of Science, and the Cochrane Library, on July 13th, 2018. Eligible studies were randomized controlled trials (RCT) in adults (patients ≥18

years) with a follow-up duration of at least one year, that included at least 10 patients per trial arm, and reported on changes in eGFR and/or proteinuria. The intervention of interest was statin therapy, the comparator either another statin, no intervention, cholesterol lowering diet, or placebo. In the entire manuscript, control treatment refers to any non-statin intervention. Combination therapy of statin with ezetimibe was also considered. A detailed outline of the search strategy is provided in the Supplemental Data, Appendix. Titles and abstracts were screened and relevant articles were read in full by two reviewers (KE and EH). Conference abstracts were excluded. No language restrictions were imposed. Post-hoc analyses of RCTs were only included when outcomes according to the original randomization group could be derived. In case of duplicate publications, we selected the publication that reported the data of interest most completely. References of included studies were additionally screened for relevant RCTs. We reported the results according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines for network meta-analyses.[10] The protocol for this meta-analysis was registered at PROSPERO: registration number CRD42018099613.[11]

Outcome measures

The outcomes of interest were annual change of estimated glomerular filtration rate (eGFR) and proteinuria. Kidney function estimates calculated by the Cockroft-Gault formula, the Modification of Diet in Renal Disease (MDRD) formula, or Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation were pooled. If change of kidney function or proteinuria was not reported, it was calculated by subtracting the baseline value from follow-up. The standard deviation (SD) of change was calculated using the SDs of eGFR or proteinuria at baseline and follow-up, according to the following formula:[12]

$$\mathrm{SD}_{change} = \sqrt{{\mathrm{SD}_0}^2 + {\mathrm{SD}_1}^2 - (2 * \ Corr * \mathrm{SD}_0 * \mathrm{SD}_1)}$$

Where SD_0 and SD_1 represent the SD of baseline and follow-up, respectively, and *Corr* represents a correlation coefficient, which describes the similarity between baseline and follow-up measurements. The correlation coefficient was derived from studies that reported both baseline and follow-up eGFR or proteinuria with an SD, and change in eGFR or proteinuria with SD, according to the following formula:[12]

$$Corr = \frac{{\mathrm{SD}_0}^2 + {\mathrm{SD}_1}^2 + {\mathrm{SD}_{change}}^2}{2 * \mathrm{SD}_0 * \ \mathrm{SD}_1}$$

Based on data from three intervention studies investigating the effect of statins on kidney function, and data from the Alpha Omega Trial, we assumed a correlation coefficient between baseline and follow-up eGFR of 0.8.[13–16] In the main analysis we compared change of eGFR or proteinuria after 12 months for statin users *vs* control treatment. If no data were reported on change in eGFR or proteinuria after one year, we used the available data to calculate an annual change assuming a linear decline in line with the results of a recent study.[17]

Data extraction and quality assessment

Data extraction was performed by two independent reviewers (KE and EH) who used a standard form. Discrepancies were resolved by discussion or by consulting a third reviewer (OD). We extracted the following data: study name, study year, trial acronym, duration, population type, treatment arms, sample size, mean age, sex (% males), diabetes (%), hypertension (%), mean systolic and diastolic blood pressure, use of renin-angiotensin system (RAS) blocking drugs (%), low-density lipoprotein (LDL) level at baseline and follow-up, baseline and follow-up eGFR, change in eGFR, baseline and follow-up proteinuria, and change in proteinuria. When the outcome of interest was not reported in a table or text, we extracted the exact numbers from figures.

The Cochrane Collaboration Risk of Bias tool was used to assess potential sources of bias: selection, performance, detection, attrition and reporting bias.[18] We scored per included RCT each type of bias as follows: low, high, or unclear risk of bias. Risk of bias was scored high in case of broken randomization, absent blinding of participants, absence of allocation concealment, and in case of large number of missing outcome data, or exclusion of patients. Since the outcome of interest was based on laboratory measurements, we considered for all RCTs, including the open-label RCTs, the risk of bias "low" with regard to blinding of outcome assessment.

Statistical analysis

First, we performed a pairwise random-effects meta-analysis for the effect of statin *vs* control on eGFR and proteinuria decline. For eGFR decline we used the weighted mean difference (WMD) as measure for the pooled estimates. For proteinuria we estimated standardized mean differences (SMD) to account for different methods to express proteinuria: urinary albumin to creatinine ratio, urinary protein excretion, urinary albumin excretion, or log-transformed protein excretion. Statistical heterogeneity was assessed by the I^2-statistic, which quantifies the variation across studies due to heterogeneity rather than chance.[19] We used meta-regression to evaluate whether heterogeneity could be explained by age, sex, diabetes, blood pressure, baseline LDL, change in LDL,

or risk of bias. Finally, we assessed the presence of publication bias visually with a funnel plot and formally by the Egger's test.[20,21] This rank-based method estimates the number and outcomes of missing unpublished studies, and adjusts the estimate after incorporating these theoretical studies.

Second, we performed a random-effects network meta-analysis, following a frequentist approach. In case multiple dosages were reported, we analyzed high and low statin dosages as separate treatments. We took as outcome the WMD of annual kidney function decline and change of proteinuria expressed as SMD. We checked for transitivity and consistency. Transitivity was judged clinically; consistency was judged formally.[22] We tested for possible inconsistency globally using a χ^2-test, and locally by calculating inconsistency factors for each comparison in closed loops. In case of minor inconsistencies, possible reasons for inconsistency were considered. Furthermore, we estimated for each statin, compared to control, the treatment effect with 95%-confidence intervals and prediction intervals. The prediction interval represents the expected range of true effects in similar (future) studies, and will be broader than the confidence interval in case of high heterogeneity.[23] Finally, for each statin, with or without ezetimibe, we calculated the surface under the cumulative ranking (SUCRA) line. We used the SUCRA to provide a hierarchic overview of treatments, and to give an impression of the most efficacious treatments.[24] The SUCRA takes into account for every treatment the cumulative probabilities of all possible rankings. If a treatment always ranks first, the SUCRA is 100% (or 1), and 0% (or 0) if it always ranks last.[25]

We repeated the analyses excluding RCTs with a total sample size <100 patients or stratified by open-label (yes/no) or post-hoc (yes/no) status. Subgroup analyses were not considered if too few RCTs remained to form a network. All statistical analyses were performed using STATA Statistical Software version 14 (Statacorp, Texas, USA), and the *StataNMA* package.[26]

RESULTS

Characteristics of included studies

After removing duplicate RCTs, 1303 titles and abstracts were screened for eligibility; 76 full publications were assessed. Finally, 43 RCTs comprising over 110,000 patients reported in 42 publications were included (Figure 1). Of these 42 publications, 40 were in English, one was Russian,[27] and one Japanese.[28] In total, 40 RCTs reported about the effect of statins on change of eGFR,[13-15,27,29-63] of which 30 compared a statin to control, and 10 compared two or more statins with each other. The effect of statins on proteinuria was reported in 25

RCTs,[13,14,28,29,32-34,36,39,45,46,48-54,57,60,62-65] of which 19 compared a statin to control intervention, and six compared two or more statins. Characteristics of included RCTs are shown in Table 1. The included RCTs investigated seven different statins with varying dosages, and in three RCTs a statin was combined with ezetimibe.[40,46,48] Of all included RCTs, 11 comprised coronary heart disease patients, 11 comprised CKD patients, and 11 comprised diabetes mellitus type 2 patients. The mean age of the enrolled patients in most RCTs was over 50 years and about 66% were men. The unweighted mean (range) of baseline LDL-cholesterol from all individual RCTs was 3.7 (2.2-7.8) mmol/L, and statin compared to control treatment led to a mean (SD) 27% (9%) reduction of the serum LDL level. The majority of RCTs had a low risk of bias (Supplementary Figure S1). However, about a 44% of all RCTs was open-label and about 25% were post-hoc analyses.

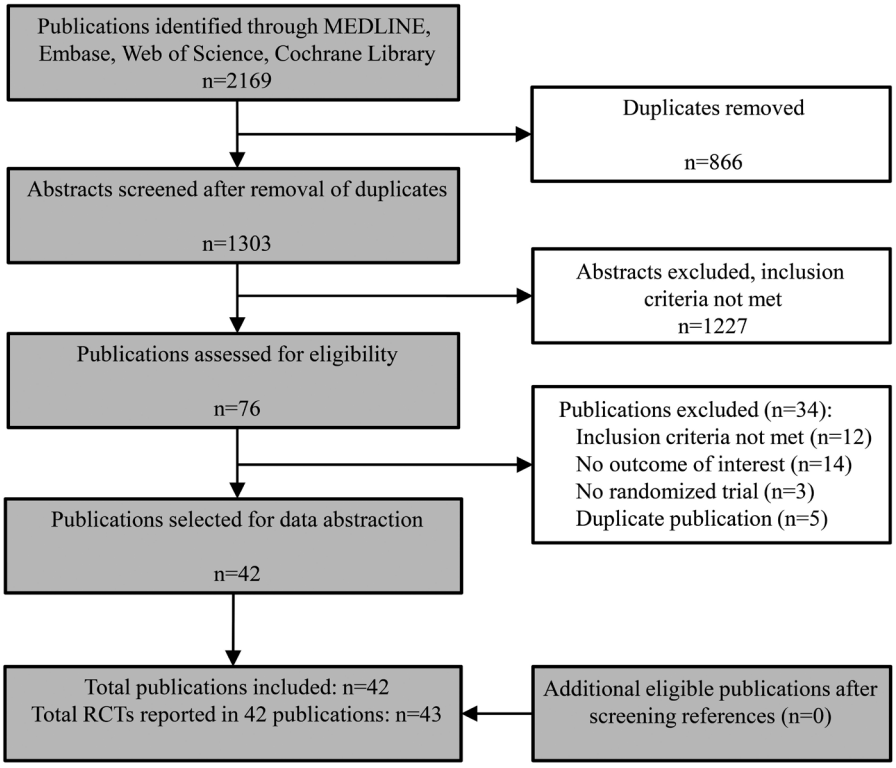

Figure 1: Flow chart of literature search and included full text publications. All included publications were included in quantitative analyses, depending on the reported endpoint(s).

Table 1: characteristics of included studies.

Author, year Study name	Population	Intervention	Sample size (n)	Follow-up (y)	Mean baseline characteristics per RCT						Outcome [annual change (SD)] per intervention	
					Age (y)	Male sex (%)	Diabetes (%)	Blood pressure (mmHg)	eGFR (mL/min/1.73m^2)	LDL (mmol/L)	eGFR	Proteinuria (measure)
Abe, 2015	CKD	Rosuvastatin 2.5 mg Pitavastatin mean1.4 mg	134	1	70	58	44	.	58	3.6	2.80 (12.1) 0.90 (13.8)[a]	-392 (802) -250 (707)[a] (UACR)
Amarenco,2014 SPARCL	Stroke, TIA	Atorvastatin 80 mg Placebo	4719	5	63	60	17	139/82	66	3.4	0.96 (13.1) -0.50 (13.1)[b]	.
Athyros, 2004 GREACE	CHD	Atorvastatin mean24 mg Control	1600	4	58	78	20	123/75	77	.	2.00 (2.0) -0.75 (1.8)[a]	.
Atthobari, 2006 PREVEND-IT	General population	Pravastatin 40 mg Placebo	788	4	52	66	3	131/76	76	4.1	0.15 (3.7) -0.25 (1.9)[a]	-0.02 (0.07) -0.03 (0.08)[ac] (UAE)
Bianchi, 2003	CKD	Atorvastatin 40 mg Placebo	56	1	56	47	0	133/85	50	5.1	-1.00 (5.9) -5.80 (6.0)[a]	-1.0 (0.47) 0.3 (0.47)[a] (UPE)
Castelao, 1993	Transplant	Lovastatin 20 mg Simvastatin 10 mg	51	1	44	69	.	.	52	4.9	-1.00 (16.6) -4.60 (15.3)	0.38 (1.9) 0.31 (1.1) (UPE)
Colhoun, 2009 CARDS	DM2	Atorvastatin 20 mg Placebo	2838	4	62	68	100	144/83	64	3.0	0.48 (2.7) 0.30 (2.6)	.
Dalla Nora, 2003	DM2	Atorvastatin 10 mg Placebo	25	1	65	60	100	.	.	3.5	.	2.0 (1.9) 6.0 (1.9)[d] (AER)
Deedwania,2015 SAGE	CAD	Atorvastatin 80 mg Pravastatin 40 mg	868	1	72	69	23	.	62	3.8	2.38 (10.4) 0.18 (10.3)[b]	.
Fassett, 2010	ADPKD	Pravastatin20 mg Control	60	2	51	39	.	133/86	55	3.3	-0.31 (10.4) -1.34 (12.2)	-0.04 (0.20) 0.01 (0.09) (UPE)
Fassett, 2010 LORD	CKD	Atorvastatin 10 mg Placebo	132	3	60	65	8	143/81	31	3.4	-1.04 (3.84) -1.47 (3.74)	-0.39 (0.71) -0.14 (0.85) (UPE)
Fellstrom, 2004 ALERT	Transplant	Fluvastatin 40 mg Placebo	439	5	50	66	19	144/86	52	4.1	-0.93 (8.9) -1.87 (8.3)[a]	.
Fried, 2001	DM1	Simvastatin 10 mg Placebo	39	1.5	32	56	100	.	.	3.3	.	0.09 (0.44) 0.14 (0.66)[d] (AER)

Table 1: Continued

Author, year Study name	Population	Intervention	Sample size (n)	Follow-up (y)	Mean baseline characteristics per RCT: Age (y)	Male sex (%)	Diabetes (%)	Blood pressure (mmHg)	eGFR (mL/min/1.73m²)	LDL (mmol/L)	Outcome [annual change (SD)] per intervention: eGFR	Proteinuria (measure)
Gheith, 2002	Nephrotic syndrome	Fluvastatin 20 mg Control	43	1	23	42	.	.	107	7.8	-4.80 (28.8) -35.4 (29.4)[a]	-6.0 (2.3) -2.0 (2.4)[de] (UPE)
Haynes, 2014 SHARP	CKD	Simvastatin 20 mg/eze Placebo	5037	4	63	62	23	139/80	27	2.9	-1.66 (3.5) -1.83 (3.5)	.
Holme, 2010 IDEAL	MI	Atorvastatin 80 mg Simvastatin 20 mg	8888	4.8	62	81	12	137.80	68	3.1	0.01 (2.7) 0.34 (2.7)	.
HPS, 2003 HPS	DM	Simvastatin 40 mg Placebo	20536	4.8	64	76	29	144/81	.	3.3	-1.23 (1.86) -1.40 (1.83)	.
Huskey, 2009 4S	CHD	Simvastatin 20 mg Placebo	3842	5.5	58	80	4	139/83	76	4.9	-0.34 (7.4) -0.41 (7.4)[f]	.
Kendrick, 2010 AFCAPS/Tex	Primary prevention	Lovastatin 20 mg Placebo	4994	5.3	58	85	2	138/78	87	3.8	-1.30 (3.5) -1.40 (3.5)	.
Kimura, 2017 ASUCA	CKD	Atorvastatin 5-20 mg Control	334	2	63	64	34	133/77	55	3.7	-1.15 (4.4) -1.30 (4.4)	0.3 (1.3) -0.2 (1.3) log(UAE)
Kimura, 2012	DM2	Pitavastatin 2 mg Pravastatin 10 mg	83	1	65	57	100	132/76	74	3.4	-2.0 (9.0) -0.5 (9.5)[b]	-50 (150) 25 (175)[b] (UACR)
Kinouchi, 2013	Dyslipidemia	Fluvastatin 20 mg Fluvastatin 20 mg/eze	54	1	54	67	6	140/90	71	4.1	-4.10 (7.7) 4.10 (6.4)	22.5 (72.4) -44.7 (74.5)[d] (UAE)
Koren, 2009 ALLIANCE	CHD	Atorvastatin mean 41mg Control	2442	4.5	61	82	22	134/79	73	3.8	0.18 (6.4) -0.30 (7.2)	.
Kouvelos, 2015	Vascular surgery	Rosuvastatin 10 mg Rosuvastatin 10 mg/eze	262	1	71	90	30	.	65	3.8	-7.60 (10.1) -6.80 (10.7)[a]	0.9 (2.0) 0.5 (1.9)[ad] (UPE)
Lam, 1995	NID-DM	Lovastatin 20-40 mg Placebo	34	2	56	56	100	.	84	4.2	-1.10 (5.7) -1.30 (3.6)[ab]	0 (0.1) 0.25 (0.2)[ab] (UPE)
Lee, 2005	Controlled HT	Pravastatin 10 mg Placebo	61	1	49	68	0	121/73	87	3.2	13.0 (13.3) 4.0 (12.4)[a]	-673 (448) -7 (327)[ab] (UPE)
Lemos, 2013	CKD	Rosuvastatin 10 mg Control	77	2	58	61	21	.	40	3.1	-1.15 (6.0) -2.50 (5.1)a	0.08 (0.18) 0.23 (0.26)[ad] (UPE)

Table 1: Continued

Author, year Study name	Population	Intervention	Sample size (n)	Follow-up (y)	Mean baseline characteristics per RCT						Outcome [annual change (SD)] per intervention	
					Age (y)	Male sex (%)	Diabetes (%)	Blood pressure (mmHg)	eGFR (mL/min/1.73m^2)	LDL (mmol/L)	eGFR	Proteinuria (measure)
Mori, 1992	NID-DM	Pravastatin 10 mg Control	33	1	63	36	100	134/80	.	2.9	.	-50.5 (54.7) -5.4 (71.8)[a] (UACR)
Mou, 2016	Chronic glom. nephritis	Pravastatin 20 mg Control	48	1.8	51	.	8	133/75	75	3.5	-1.08 (12.7) -4.33 (10.6)[ab]	-0.33 (0.9) -0.27 (0.9)[ab] (UPE)
Nanayakkara, 2007 ATIC	CKD	Pravastatin 40 mg * Placebo	87	2	53	57	0	135/79	34	3.6	0 (4.3) 0.15 (4.3)[ab]	-0.1 (0.8) 0.2 (0.8)[a] log(UAE)
Ohsawa, 2015	CKD	Pitavastatin 1-4 mg Control	28	1	62	71	33	130/78	49	3.6	-3.50 (3.21) -4.20 (2.96)[a]	-244 (574) -338 (1141)[a] (UACR)
Rahman, 2008 ALLHAT	HT, HCh	Pravastatin 40 mg Control	10355	6	67	51	35	143/83	78	3.8	-1.45 (5.9) -1.65 (5.9)[a]	.
Rutter, 2011 PANDA	DM2	Atorvastatin 80 mg Atorvastatin 10 mg	119	2.5	64	83	100	.	67	3.1	1.0 (13.8) -3.0 (11.8)[ab]	.
Sawara, 2008	CKD	Rosuvastatin 2.5 mg Control	38	1	65	0	.	127/78	53	3.3	2.60 (12.3) -2.20 (10.6)[a]	-0.04 (0.19) 0.05 (0.24)[a] (UPE)
Scanferla, 1991	CKD	Sim/pravastatin 10 mg Control	24	1	54	58	.	172/106	40	.	-1.80 (4.2) -3.10 (4.2)	.
Shepherd, 2007 TNT	CAD	Atorvastatin 80 mg Atorvastatin 10 mg	10001	5	61	81	15	131/78	65	2.5	1.5 (9.7) 0.1 (9.7)[bf]	.
Takazakura, 2015	DM	Atorvastatin 10 mg Pravastatin 10 mg Control	106	1	62	87	100	129/0	64	3.0	-0.80 (11.4) -2.80 (10.8) -3.10 (9.6)[a]	-0.2 (0.4) -0.1 (0.7) 0.1 (0.5)[a] log(UACR)
Tonelli, 2005 PPP **	CAD	Pravastatin 40 mg Placebo	18569	5	58	90	7	133/81	73	4.2	Effect of pravastatin: 0.10 (0.02; 0.17) mL/min/1.73m^2 [g]	.

Table 1: Continued

Author, year Study name	Population	Intervention	Sample size (n)	Follow-up (y)	Mean baseline characteristics per RCT						Outcome [annual change (SD)] per intervention	
					Age (y)	Male sex (%)	Diabetes (%)	Blood pressure (mmHg)	eGFR (mL/min/1.73m²)	LDL (mmol/L)	eGFR	Proteinuria (measure)
Vidt, 2011 JUPITER	Healthy population	Rosuvastatin 20 mg Placebo	16279	2.3	66	62	31	.	75	.	-7.10 (11.9) -7.70 (11.8)	.
Yakusevich, 2013	Stroke	Simvastatin 40 mg Control	210	1	66	45	.	.	76	2.2	7.05 (12.1) 1.37 (13.8)[f]	.
Yasuda, 2004	CKD	Fluvastatin 20 mg Control	80	0.9	58	46	43	144/80	60	4.4	-8.67 (3.9) -6.50 (4.0)[a]	0 (0.14) 0 (0.15)[a] (UAE)
De Zeeuw, 2015 PLANET I	DM	Rosuvastatin 10 mg Rosuvastatin 40 mg Atorvastatin 80 mg	325	1	58	70	100	139/79	71	3.9	-3.70 (14.7) -7.29 (20.4) -1.61 (13.0)	2 (79) -4 (77) -13 (57) %change
De Zeeuw, 2015 PLANET II	Non-DM proteinuria	Rosuvastatin 10 mg Rosuvastatin 40 mg Atorvastatin 80 mg	220	1	49	62	0	130/81	75	4.3	-2.71 (13.3) -3.30 (12.5) -1.74 (14.2)	-6 (99) 8 (75) -24 (60)

ACS, acute coronary syndrome; ADPKD, autosomal dominant polycystic kidney disease; CAD, coronary artery disease; CHD, coronary heart disease; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; eze, ezetimibe; HT, hypertension; MI, myocardial infarction; TIA, transient ischemic attack; (NID-)DM1/DM2, non-insulin dependent diabetes mellitus 1 or 2, LDL, low-density lipoprotein; prot, proteinuria; UACR, urinary albumin-to-creatinine ratio; UAE, urinary albumin excretion; UPE, urinary protein excretion.
*Intervention was a combination of statin and vitamin E supplementation.
**PPP: Pravastatin Pooling Project, study representing pooled estimates of three RCTs: LIPID, CARE, and WOSCOPS. Individual data on each RCT was not published.
a: based on eGFR (SD) value at baseline and follow-up. SD of eGFR change was calculated according to the formula provided in the Cochrane Handbook.[11]
b: data extracted from figure.
c: reported geometric mean was log-transformed to achieve normal distribution with symmetrical SD.
d: SD acquired by dividing interquartile range by 1.35.
e: no SD or SE reported, these were therefore borrowed from comparable studies.
f: SD of baseline eGFR value used to calculate SD of eGFR change.
g: only effect of treatment *vs* control reported.

Pairwise comparison: statins and eGFR decline

Except for two medium sized trials (Yasuda *et al.*, and Nanayakkara *et al.*), effect estimates of all RCTs showed a protective effect of statin on eGFR decline.[53,62] Random-effects meta-analysis showed that statin use, compared to control, led to a 0.61 (95% CI 0.27; 0.95) mL/min/1.73m^2 slower annual eGFR decline (Figure 2). When only RCTs with a sample size of at least 100 patients (n=16) were analyzed, the beneficial effect of statin treatment on annual eGFR decline was 0.58 (0.23; 0.92) mL/min/1.73m^2. Heterogeneity between RCTs was high, with an I^2 of 96%. Meta-regression showed that higher systolic blood pressure at baseline was significantly associated with smaller effects of statins, explaining 40% of the between-study variance. We found no evidence for interaction between diabetes and statins with regard to the beneficial effect on kidney function decline. Age, sex, serum LDL level, or change in LDL, had no significant impact on the effect estimates. In post-hoc RCTs (n=11) the beneficial effect on annual kidney function decline of statins *vs* control was smaller but more precise than in RCTs in which change in eGFR was the primary outcome (n=17): 0.55 (0.19; 0.92) *vs* 1.55 (0.26; 2.85) mL/min/1.73m^2, respectively. In open-label RCTs (n=17, mean sample size 4326) the beneficial effect on eGFR decline of statins *vs* control was stronger than in blinded RCTs (n=13, mean sample size 1161): 1.25 (0.08; 2.42) *vs* 0.23 (0.11; 0.34) mL/min/1.73m^2, respectively. The funnel plot for eGFR decline was slightly asymmetrical (Supplementary Figure S2), but the Egger's test for small study effects was not significant (p= 0.3).

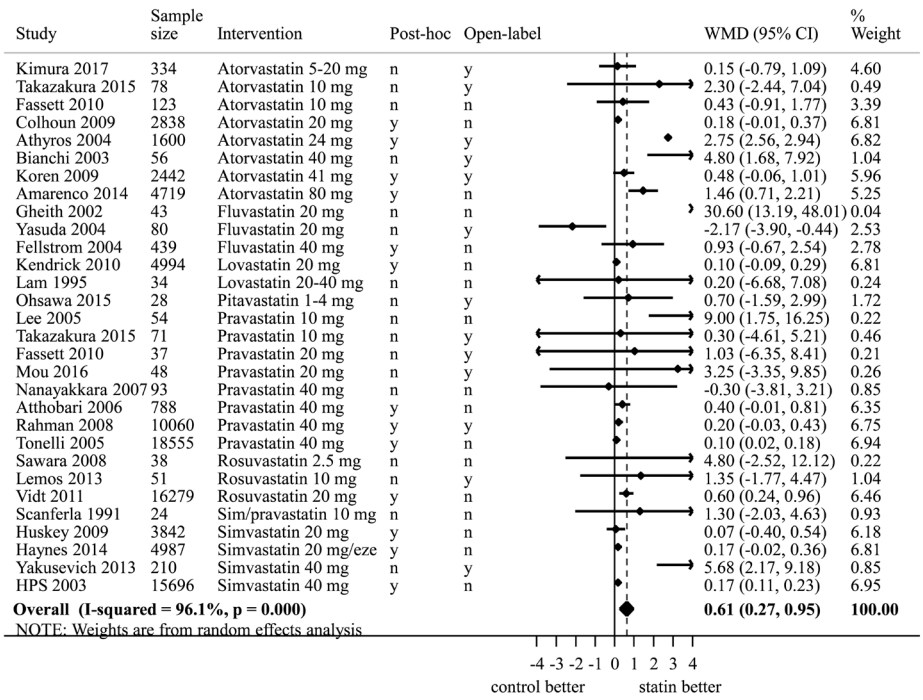

Figure 2: Pairwise random effects meta-analysis of randomized controlled trials investigating the effect of statin therapy versus control on the rate of annual eGFR decline. Positive values mean slower eGFR decline for statin users *vs* non-users, thus favouring statin use. eGFR, estimated glomerular filtration rate; eze, ezetimibe 10 mg; WMD, weighted mean difference.

Pairwise comparison: statins and proteinuria

The two largest RCTs showed that statin treatment *vs* control did not lower proteinuria: SMD of 0.40 (0.18; 0.61) and 0.18 (0.04; 0.32), respectively.[32,63] In a meta-analysis, statin use compared to control showed a significant reduction of proteinuria with an SMD -0.58 (-0.88; -0.29) (Figure 3). However, the funnel plot of the effect of statins on proteinuria suggested publication bias (Supplementary Figure S3) and the Egger's test was significant (p<0.001).

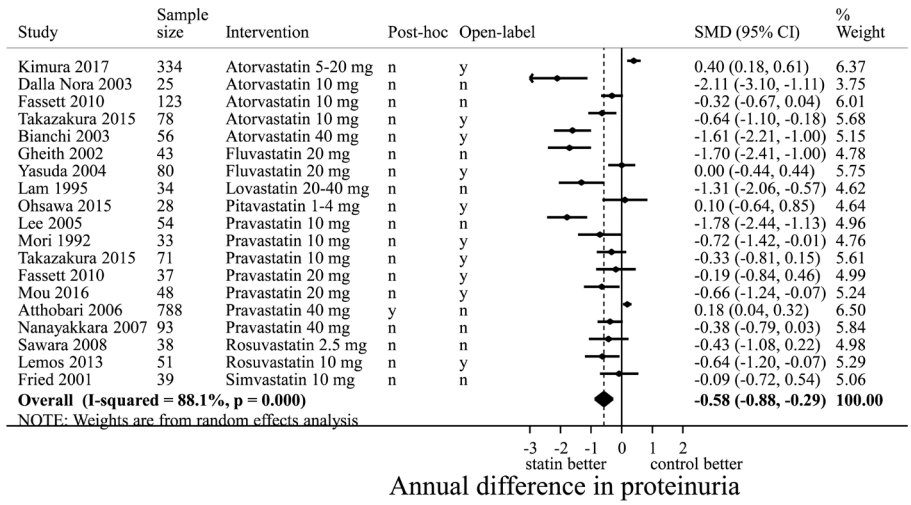

Figure 3: Pairwise random effects meta-analysis of randomized controlled trials investigating the effect of statin therapy versus control on the rate of annual change in proteinuria. Negative values mean a decrease in proteinuria for statin users *vs* non-users, thus favouring statin use. Effects expressed as SMD (standardized mean difference).

Network meta-analysis

Figure 4 (upper panel) shows the network plot of different statin treatments for change in eGFR. Each connection was formed by maximally 4 RCTs. We found no evidence for inconsistency in the network for eGFR decline and proteinuria using global tests (p-value for inconsistency 0.8) or local tests (p >0.3 for all loops). We found that almost all statins performed better than control (Figure 5). The most beneficial effect on eGFR decline was caused by fluvastatin 20 mg/ezetimibe 10 mg, rosuvastatin 20 mg/ezetimibe 10 mg, pravastatin 10-20 mg, and atorvastatin 40-80 and 10<40 mg. However, point estimates had broad 95%-confidence intervals and prediction intervals. Except for combined fluvastatin 20 mg/ezetimibe 10 mg and atorvastatin 40-80 mg, all 95%-confidence intervals crossed the line of no effect.

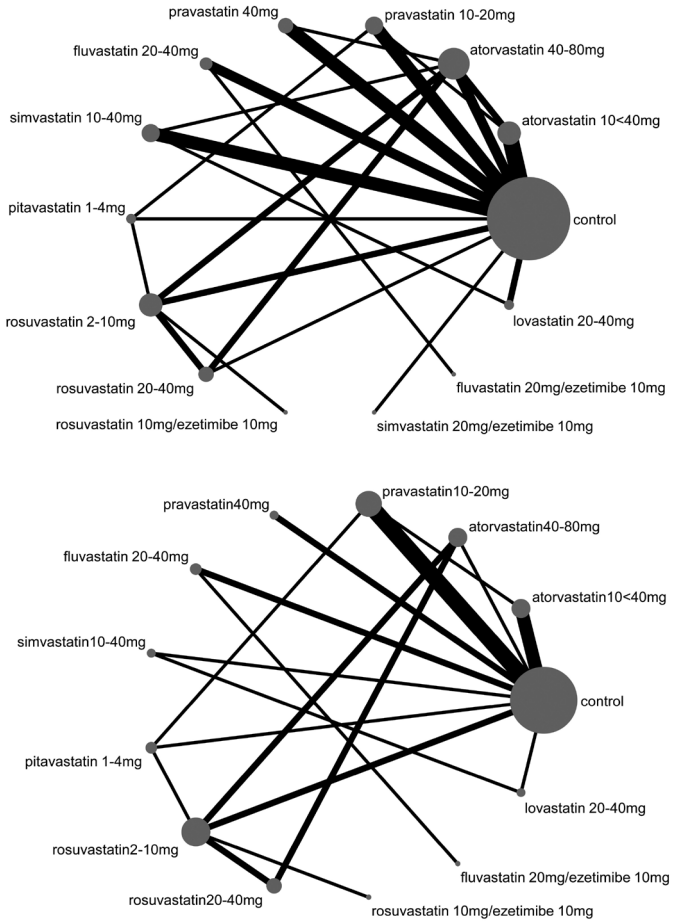

Figure 4: Network plots for outcome eGFR decline (upper panel) and proteinuria (lower panel). The width of the interconnecting lines is proportional to the number of RCTs providing evidence (ranging from 1 to 4). The size of the nodes is proportional to the total number of patients. eGFR, estimated glomerular filtration rate; RCT, randomized controlled trial.

Figure 4 (lower panel) shows the network plot for all statin treatments regarding proteinuria. For proteinuria, no single RCT compared the combination therapy simvastatin/ezetimibe. Globally, there was no evidence for inconsistency (p-value 0.8). However, using local tests, there were 2 inconsistent loops: control, atorvastatin 40-80 mg, rosuvastatin 2-10 mg (p=0.04) and control, simvastatin 10-40 mg, lovastatin 20-40 mg (p=0.03). The inconsistencies between direct and indirect effects were introduced by the relatively large effect estimates of small studies (n <60). The most efficacious treatments regarding

proteinuria were fluvastatin 20 mg/ezetimibe 10 mg, atorvastatin 40-80 mg, and rosuvastatin 20 mg/ezetimibe 10 mg (Figure 6).

Finally, SUCRA analysis showed that control treatment had the lowest SUCRA. Fluvastatin 20 mg/ezetimibe 10 mg had the highest SUCRA value for eGFR decline (99%) and fluvastatin 20 mg/ezetimibe 10 mg (86%) as well as atorvastatin 40-80 mg (78%) had the highest SUCRA value for change in proteinuria (Figure 7).

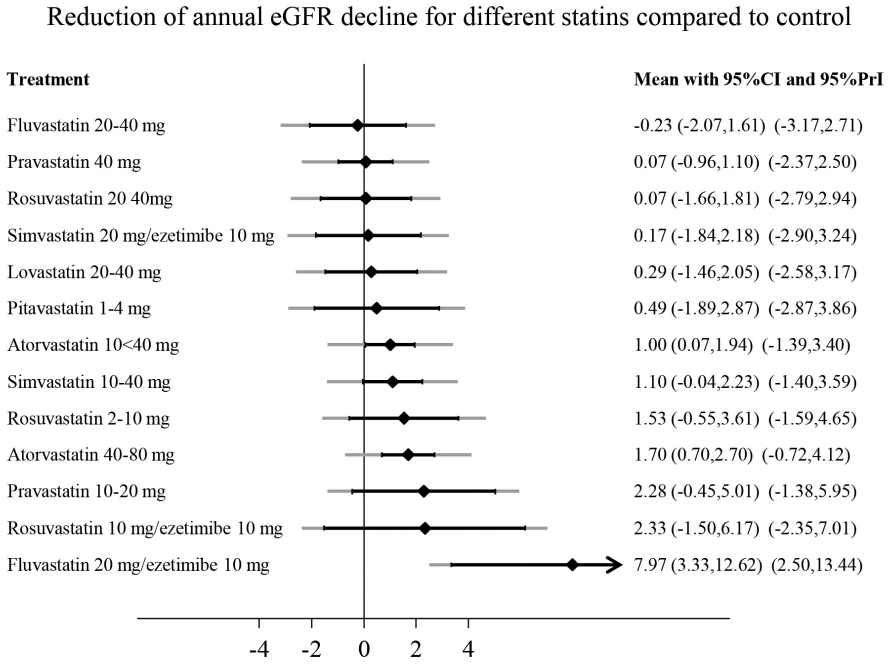

Figure 5: Effect of different statins compared to control treatment, on annual eGFR decline. Effects are presented as weighted mean differences (WMD). Positive values mean a slower eGFR decline. Black lines around point estimates reflect 95%-confidence intervals, grey lines reflect prediction intervals. Prediction intervals represent the expected range of true effects in (future) similar studies and is suitable to assess the variability of effect across different settings.
CI, confidence interval; eGFR, estimated glomerular filtration rate; PrI, prediction interval.

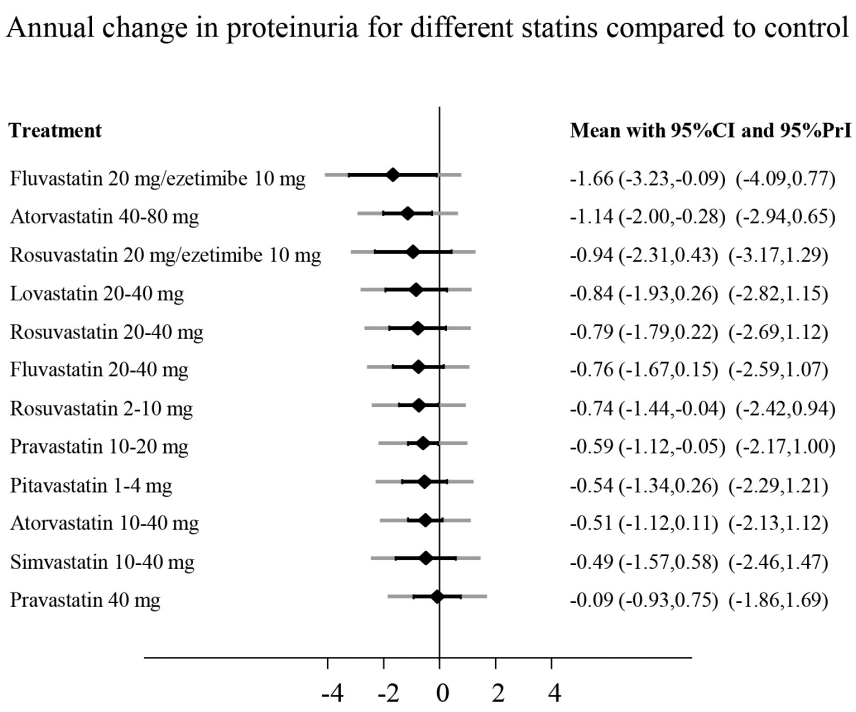

Figure 6: Effect of different statins compared to control treatment, on annual change in proteinuria. Effects are presented as standardized mean differences (SMD). Negative values mean a reduction of proteinuria. Black lines around point estimates reflect 95%-confidence intervals, grey lines reflect prediction intervals. Prediction intervals represent the expected range of true effects in (future) similar studies and is suitable to assess the variability of effect across different settings.
CI, confidence interval; eGFR, estimated glomerular filtration rate; PrI, prediction interval.

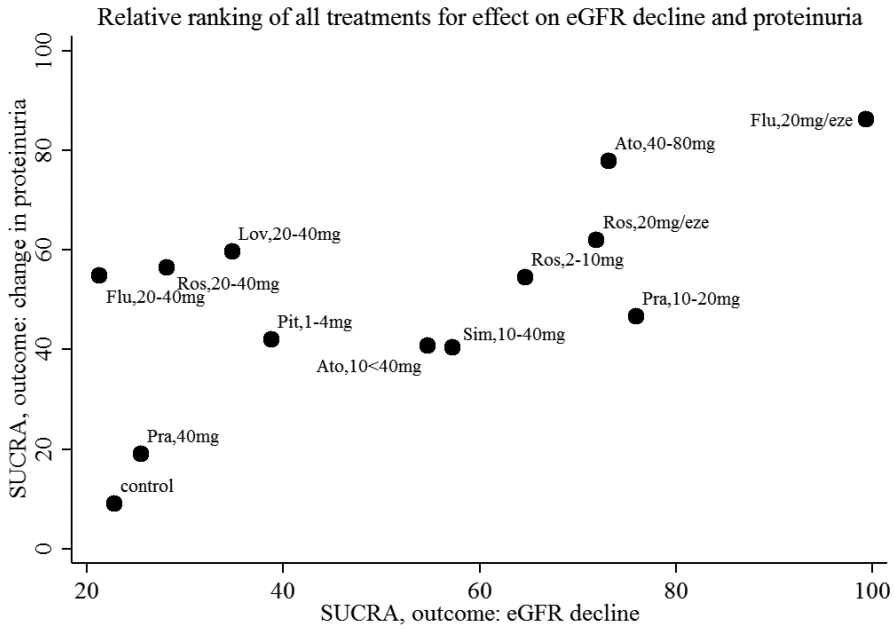

Figure 7: SUCRA analyses. Each dot represents the SUCRA value of each treatment. The SUCRA takes into account for every treatment the cumulative probabilities of all possible rankings. If a treatment always ranks first or last, the SUCRA is 100% or 0%, respectively. The horizontal axis shows SUCRA values with regards to the outcome eGFR decline, the vertical axis shows the SUCRA for the outcome proteinuria.
Ato, atorvastatin; eze, ezetimibe 10 mg; Flu, fluvastatin; Lov, lovastatin; Pit, pitavastatin; Pra, pravastatin; Ros, rosuvastatin; Sim, simvastatin; eGFR, estimated glomerular filtration rate; SUCRA, surface under the cumulative ranking curve.

Sensitivity analyses

Since we included RCTs with seven different types of statin treatments with one or more different dosages, networks of subgroups had only few closed loops. Therefore, estimates were based mostly on either direct or indirect evidence, but not on mixed evidence. Nonetheless, we repeated the network meta-analysis for eGFR decline excluding RCTs with a sample size <100 (n=16), excluding open-label RCTs (n=17), or excluding post-hoc analyses (n=20). Although effect estimates and rankings of individual treatments were variable across the analyses, in general atorvastatin 40-80 mg, fluvastatin 20 mg/ezetimibe 10 mg, pravastatin 10-20 mg, simvastatin 10-40 mg, and fluvastatin 20 mg were the most effective treatments with regard to eGFR decline. However, 95%-confidence intervals had substantial overlap, and individual treatments were rarely statistically significantly different from control. Since only a

small number of RCTs with small sample sizes studied the effect of statins on proteinuria, we could not perform the aforementioned sensitivity analyses.

DISCUSSION

In this network meta-analysis, we showed that there are no substantial differences in the efficacy of seven different statins and dosages, with or without ezetimibe, regarding slowing down eGFR decline or reducing proteinuria. If anything, the combination of fluvastatin 20 mg/ezetimibe 10 mg and atorvastatin 40-80 mg most consistently had the strongest beneficial effect on both renal endpoints, but the differences between treatments were small and confidence intervals were wide. In the pairwise meta-analysis we showed that use of statins lowered the rate of annual kidney function decline by 0.61 mL/min/1.73m^2 and reduced the amount of proteinuria by -0.58 (-0.88;-0.29) standard deviations per year.

Our results are in line with a recent meta-analysis Su *et al.* which reported that statins compared to control led to a 0.41 (0.11; 0.70) mL/min/1.73m^2 slower annual eGFR decline and a reduction of -0.65 (-0.94; -0.37) standard deviations in proteinuria.[8] The small difference in outcomes between the present study and Su *et al.* are explained by different inclusion criteria. In contrast to the study of Su *et al.*, we included three RCTs investigating combinations of statins plus ezetimibe. Including also treatments combining statins with ezetimibe, results in a more complete review of existing literature on lipid-lowering therapy by statins. As a consequence we incorporated in our meta-analysis three extra RCTs, including the SHARP trial (n=5037). Furthermore, we excluded RCTs with a short follow-up (<12 months) or less than 10 patients per study arm, of which Su *et al* included 19 RCTs. Finally, we found that the beneficial effect of statins on eGFR decline was weaker in RCTs with a higher mean systolic blood pressure. Systolic blood pressure explained 40% of the between-study variance. Taken together, these results suggest that a high systolic blood pressure modifies the effect of statins on eGFR decline. Hypertension is most likely a stronger risk factor for kidney function decline compared to hypercholesteremia. Therefore, we speculate that the positive effect of statins on kidney function decline is overwhelmed in the presence of high blood pressure.

In our network meta-analysis, we specifically investigated the efficacy of individual statins and different dosages, using both direct and indirect evidence. We showed that each different statin compared to placebo had a beneficial effect on the annual eGFR decline and reduced proteinuria. However, confidence intervals were broad for individual treatment comparisons in our

network, due to the small number of RCTs contributing to each comparison. Su *et al.* showed in subgroup analyses the strongest beneficial effect on change in eGFR decline for atorvastatin, fluvastatin, and rosuvastatin.[8] However, they pooled for each statin all dosages. The validity of these comparisons may be limited, considering the clear differential effects of different dosages.[8,66]

We showed that fluvastatin 20 mg/ezetimibe 10 mg was the most efficacious treatment regarding both renal outcomes. However, this result was strongly influenced by the study of Kinouchi *et al.*, comprising 54 patients, reporting an annual eGFR decline of -4.1 mL/min/1.73m^2 in patients treated with fluvastatin 20 mg compared to an annual eGFR increase of 4.1 mL/min/1.73m^2 in patients treated with fluvastatin 20 mg/ezetimibe 10 mg.[46] Since the average annual eGFR decline in adults with a history of cardiovascular disease is about 2 mL/min/1.73m^2, the reported effect of Kinouchi *et al.* of 8.2 mL/min/1.73m^2 is large, and should be interpreted with caution.[67] We found that the second most efficacious statin on both renal endpoints was high dose atorvastatin, which improved the annual eGFR decline by 1.70 (0.70; 2.70) mL/min/1.73m^2 and reduced proteinuria by 1.14 (0.28; 2.00) standard deviations, compared to control.

Statins included in the present study reduced LDL levels on average by 27%, which is in line with a previous meta-analysis showing an LDL-lowering effect for all statins.[66] However, there is no clear evidence that high LDL itself increases CKD risk.[68] Statins also may have pleiotropic effects favourable for reducing CKD progression, such as lowering oxidative stress, reducing inflammation, and stabilizing atherosclerotic plaques.[7,69] Hence, current guidelines recommend a statin for patients at risk for CKD, independent of LDL levels.[9,70]

The main strength of the current study is that we performed a network meta-analysis, in addition to a pairwise meta-analysis, to investigate differential effects of different statins with or without ezetimibe. We only included RCTs because they are more likely to provide unbiased information. We excluded small trials (<10 patients per arm) since they are more susceptible to publication bias.

This network meta-analysis has several limitations. First, heterogeneity was high (I^2 = 96%) owing to variation of the included patient populations across RCTs, differences in blinding methods, randomization procedures, sample size, and variability in primary endpoints. The I^2 statistic represents statistical heterogeneity, rather than clinically relevant heterogeneity, and is most strongly affected by the sample size of the individual studies. Upon increasing precision (sample size) of studies within a meta-analysis, the I^2 statistic rapidly approaches 100%.[71] Deciding whether it is valid to pool studies, should be based on the clinical relevance of any present heterogeneity,

rather than solely on the I^2 statistic.[71] We used random effects models to take heterogeneity into account. Second, we found an asymmetric funnel plot regarding proteinuria, which may be an indication of publication bias. On the other hand, larger compared to smaller RCTs showed a weak but opposite effect. Thus, the asymmetry may also be the consequence of inclusion of smaller RCTs with lower quality. Therefore, we cannot rule out that the beneficial effect of statins on proteinuria is an overestimation. Additionally, there were relatively few RCTs investigating the effect of statins on proteinuria, and most of them were small (sample size <100). Small studies therefore had a large impact on the network meta-analysis estimates, introducing inconsistencies especially in loops comprising small numbers of RCTs. The advantage of a network analysis is that it takes both direct and indirect effects into account, reducing the impact of single studies with a small sample size. For the outcome eGFR decline, the sample sizes of the included RCTs were large (24 RCTs with n>100) which improved precision and reduced potential publication bias. The much smaller effect of statins compared to control in double blind compared to open-label RCTs may suggest bias due to the lack of blinding in the open-label RCTs. Since 17 out of 30 RCTs were open-label, we may have overestimated the beneficial effect on eGFR decline of statins compared to control. Third, due to the low number of RCTs contributing to each connection in the network meta-analyses, there was insufficient power to detect differences between statins. Fourth, a large number of the included RCTs used the MDRD formula to estimate eGFR, which is known to underestimate the true eGFR for values reported higher than 60 mL/min/1.73m^2.[72] If anything, this may have underestimated the beneficial effect of statin use compared to control in studies with a mean eGFR higher than 60 mL/min/1.73m^2.

In conclusion, we found a beneficial effect of different statins, with or without ezetimibe, compared to control on progression of eGFR decline, and possibly proteinuria. Due to the imprecision of individual comparisons, results were inconclusive as to which statin performs best with regard to renal outcome.

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DISCLOSURES

The authors have no disclosures to report.

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AUTHORS' CONTRIBUTIONS

Research idea and study design: KE, OD, EH; data acquisition: KE, EH; data analysis/interpretation: EH, OD, KE, JF, FD; statistical analysis: KE, OD; supervision and mentorship: JF, EH, OD, FD. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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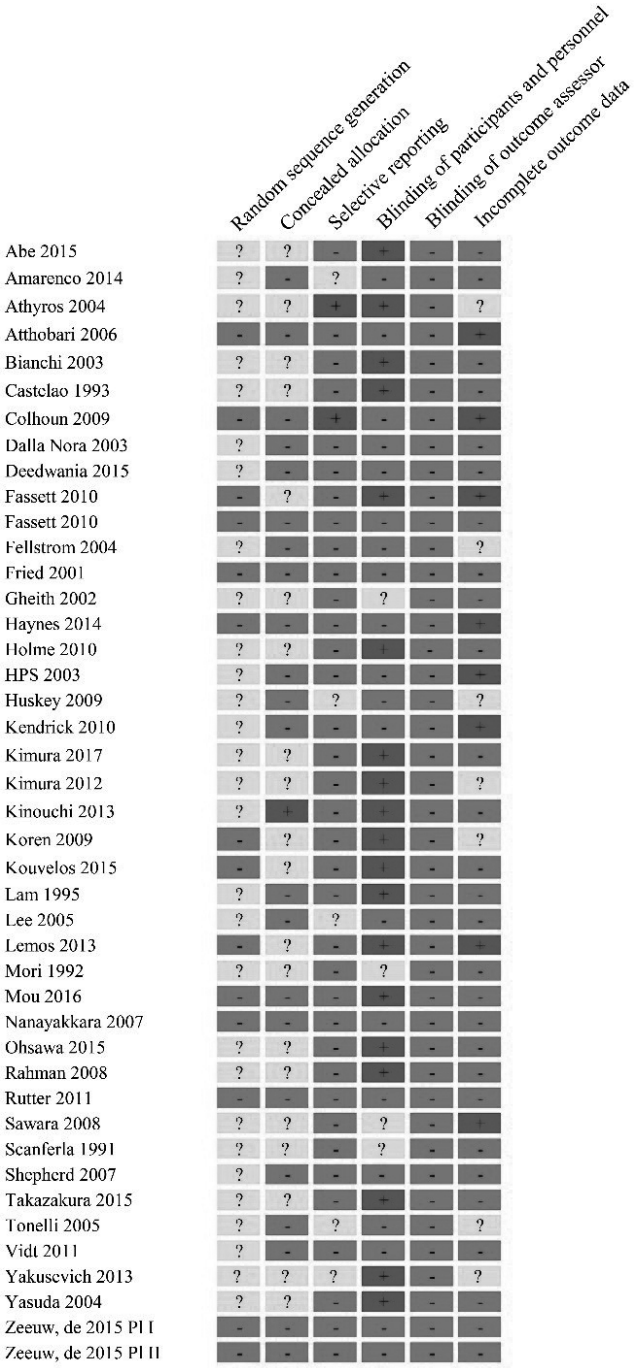
SUPPLEMENTARY DATA



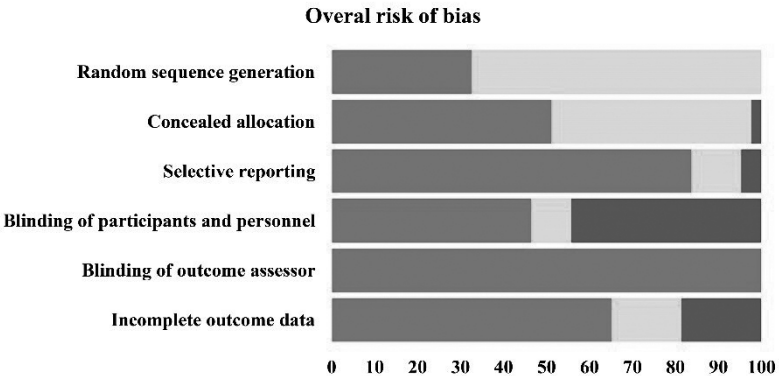


Figure S1: Risk of bias assessment per study (upper panel, page 157) and summarized over all studies (lower panel), according to the Cochrane Risk of Bias tool. Red, green and yellow cells mean high, low, and unclear risk of bias, respectively. Pl I and Pl II refer to PLANET I and II trials, respectively.

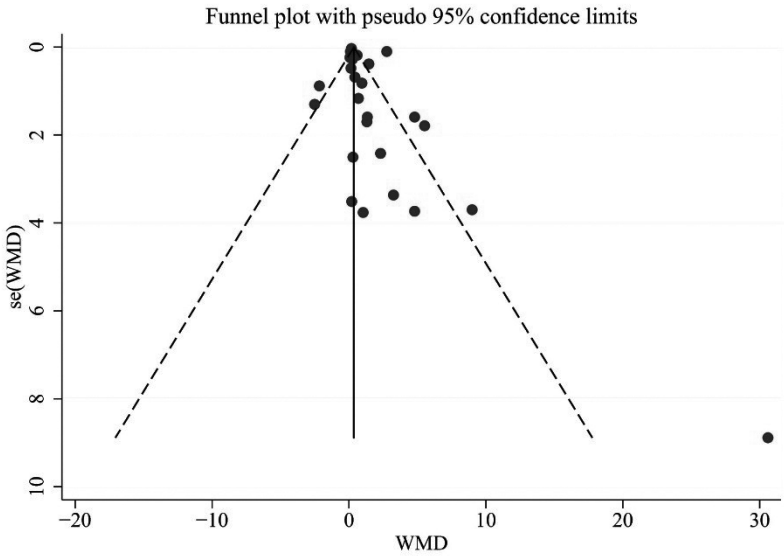


Figure S2: Funnel plot of included randomized controlled trials investigating the effect of statin therapy on annual eGFR decline. According to Egger's test there was no evidence for publication bias (p = 0.3).
eGFR, estimated glomerular filtration rate; WMD, weighted mean difference.

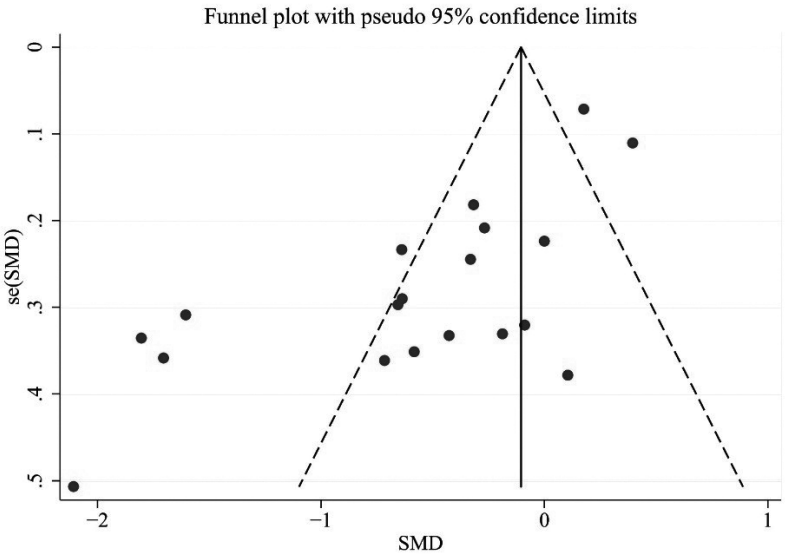


Figure S3: Funnel plot of included randomized controlled trials investigating the effect of statin therapy on change in proteinura. According to Egger's test there was significant evidence for publication bias (p <0.001).
SMD, standardized mean difference.

Chapter 7 –

The predictive value of TIMP-2 and IGFBP7 for kidney failure and 30-day mortality after elective cardiac surgery

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Submitted

ABSTRACT

Background: Acute kidney injury (AKI) is an important risk factor for chronic kidney disease, renal replacement therapy (RRT), and mortality. However, predicting AKI with currently available markers remains problematic. We assessed the predictive value of urinary tissue inhibitor of metalloprotease-2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7) regarding the need for RRT, and 30-day mortality, in elective cardiac surgery patients.

Methods: In 344 consecutive elective cardiac surgery patients, we measured urinary TIMP-2 and IGFBP7 and serum creatinine at baseline and directly after surgery. Discrimination of both urinary biomarkers was assessed by the C-statistic. Model improvement for each biomarker when added to a basic model containing serum creatinine and duration of surgery was tested by the net-reclassification index (cf-NRI) and integrated discrimination index (IDI).

Results: At baseline, mean age was 66 years and 67% were men. Of all patients, 22 required RRT following surgery. IGFBP7 pre- and post-surgery and change in TIMP-2 during surgery predicted RRT with a C-statistic of about 0.80. However, a simple model including baseline serum creatinine and duration of surgery had a C-statistic of 0.92, which was improved to 0.93 upon addition of post-surgery TIMP-2 or IGFBP7, with statistically significant cf-NRIs but non-significant IDIs. Post-surgery TIMP-2 and IGFBP predicted 30-day mortality, with C-statistics of 0.74 and 0.80.

Conclusions: In elective cardiac surgery patients, pre- and peri-operative clinical variables were highly discriminating about which patients required RRT after surgery. Nonetheless, in elective cardiac surgery patients, urinary TIMP-2 and IGFBP7 improved prediction of RRT and 30-day mortality post-surgery.

INTRODUCTION

Acute kidney injury (AKI) is an important risk factor for chronic kidney disease, need of renal renal replacement therapy (RRT) and mortality.[1,2] AKI is frequently caused by medical interventions and their side effects, such as treatment with nephrotoxic medication or peri-operative hypotension.[3] In particular, patients undergoing cardiac surgery are at high risk of AKI. The diagnosis of AKI is based on a rise in serum creatinine and/or reduction of urinary output, according to the RIFLE criteria.[4] However, usefulness of both parameters is limited in the early stages of AKI and to identify patients at risk for AKI.[5] As a consequence, AKI is often diagnosed after irreversible renal damage has already occurred. Alternative markers, which have the potential to identify patients at high risk of AKI before cardiac surgery or start of nephrotoxic medication to escalate preventive measures, are thus needed.

Recently, two urinary cell-cycle arrest markers, tissue inhibitor of metalloprotease-2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7), were approved by the U.S. Food and Drug Administration for clinical AKI prediction. Urinary levels of TIMP-2 and IGFBP7 increase upon acute kidney damage, owing to changes in tubular filtration, reduction of reabsorption, and leakage due to tubular damage.[6] TIMP-2 is preferentially secreted by distal tubule cells, while IGFBP7 is mainly secreted by proximal tubule cells.[7] The differential secretion localization of both biomarkers may characterize the extent and mechanism of kidney damage. Multiple studies showed good predictive performance of both biomarkers for AKI, in heterogeneous intensive care populations.[8]

The value of both biomarkers to predict AKI, need for RRT or death in elective cardiac surgery patients, is unclear. Therefore, we aimed to validate the predictive value of IGFBP7 and TIMP-2 regarding risk of severe AKI needing RRT. Since occurrence of AKI also increases mortality risk and may lead to longer hospital admissions, we investigated as secondary outcomes the predictive value of both biomarkers with respect to length of intensive care unit (ICU) stay and 30-day mortality.

METHODS

Participants

This single-center observational study was performed at the Leiden University Medical Center, The Netherlands. The original cohort included 814 consecutive patients aged ≥18 years, undergoing elective cardiac surgery, between

December 2006 and August 2010, as previously described in detail.[9] Exclusion criteria were pregnancy, active infection, and emergency surgery. After cardiac surgery, patients stayed in the ICU for post-operative care, according to usual clinical practice. After extubation and when hemodynamic and respiratory stable, patients were transferred to the thoracic surgery ward. For the present study, we selected all patients who stayed in the ICU for ≥48 hours (n=187) after cardiac surgery, and for whom a pre-operative plasma sample was available (92%; n=172). We considered those who stayed <48 hours in the ICU as patients with a fast and relatively uncomplicated recovery from elective cardiac surgery (n=627). For efficiency reasons, we randomly selected only 172 (28%) of these 627patients for urinary biomarker assessment. Patients staying <48 hours in the ICU were chronologically matched with patients who stayed ≥48 hours, to account for calendar time effects. The final analysis thus included 344 patients (Figure 1). Analyses were weighted towards the distribution of time of stay in the ICU of the original cohort. Demographic and medical data, including the score of the European System for Cardiac Operative Risk Evaluation (EuroSCORE) model, were obtained from electronic medical records. The EuroSCORE model was developed in 1999 and is a validated prognostic scoring model to assess mortality risk after cardiac surgery.[10] The EuroSCORE reasonably predicted development of any stage of any AKI (C-statistic 0.70) and AKI stage 3 (C-statistic 0.78) in a cohort of 440 cardiac surgery patients.[11] The score consists of 17 items, including age, gender, chronic pulmonary disease, previous cardiac surgery, serum creatinine, left ventricular dysfunction, and whether a procedure was elective or an emergency. This study was conducted in accordance with the Helsinki protocol and standard of Good Clinical Practice, and was approved by the Medical Ethics Committee of Leiden. All participants gave their written informed consent.

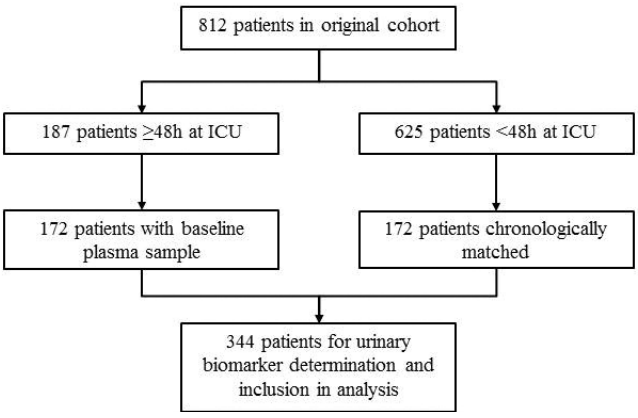

Figure 1: Flow chart of 344 patients available for analysis. ICU, intensive care unit.

Urinary measurements

Urine was sampled directly before surgery (baseline), upon admission to the ICU, 24 hours after admission to the ICU and 48 hours after admission to the ICU. After sampling, urine was partitioned into 2 mL aliquots and stored at -80 ºC until analysis. Urine sampling was only done in the ICU and stopped when the patient was transferred to the ward.

Urinary TIMP-2 and IGFBP7 were quantified using sandwich enzyme-linked immunosorbent assays according to manufacturer's instructions (ELISA, Cat. Nr. DTM200, R&D systems, Minneapolis, MN for TIMP-2, and Cat. Nr. EK0991, Boster Biological Technology, Pleasanton, CA for IGFBP7, respectively). Concentrations of urinary TIMP-2 and IGFBP7 after sample dilution were within the linear range. Low and high-level quality control (IQC) urine samples were prepared from pooled urine by spiking and analyzed in triplicate on each sample plate to assess the stability of the assay. Analysis for TIMP-2 was done with 3 different reagent lot numbers from April 2016 until October 2018. Mean TIMP-2 values (SD, %CV) of low and high IQC were 185 pmol/L (8 pmol/L, 4.4%, n=45) and 257 pmol/L (22 pmol/L, 8.7%, n=45), respectively. Analysis for IGFBP7 was done with 4 different reagent lot numbers, from April 2016 until October 2018. Mean IGFBP7 values (SD, %CV) of low and high IQC were 951 pmol/L (162 pmol/L, 17.0%, n=70) and 2231 pmol/L (370 pmol/L, 16.6%, n=70), respectively.

Osmolality was measured using Osmo-Station, ARKRAY Inc., Kyoto, Japan. We used normal, level 1, 376, and abnormal, level 2, 377 Lyphochek (BIO-RAD, Irvine, CA) quantitative urine controls for IQC, mean (SD, %CV) values were 320 mOsmol/kg (3 mOsmol/kg, 0.8%, n=11) and 868 mOsmol/kg (7 mOsmol/kg, 0.8%, n=10) for normal and abnormal quality control. Total protein (Cat. Nr 3333825190) and creatinine (Cat. Nr. 3263991190) were measured using a Cobas c502 analyzer (Roche Diagnostics, Mannheim, DE) according to the manufacturer's instructions.

Serum measurements

Serum samples were drawn immediately before cardiac surgery, upon arrival to the ICU and 24 and 48 hours after admission to the ICU. After mild centrifugation and partitioning the serum in 4 aliquots of 1 mL, serum was stored at -80 ºC until analysis. Serum cystatin C, 6600239190 and creatinine, 3263991190 were analyzed using a Cobas c502 analyzer, Roche Diagnostics, Mannheim, DE according to the manufacturer's instructions. We used Cystatin C Control Set, 63729371190, for IQC when analyzing the serum cystatin C, and mean (SD, CV%) values were 1.10 mg/L (0.02 mg/L, 1.8%, n=11), 1.57 mg/L (0.02 mg/L, 1.4%, n=12) and 4.06 mg/L (0.05 mg/L, 1.3%, n=11) for the CYSC2 Control 1, 2 and 3.

Outcomes

The primary outcome was need for RRT within two weeks after surgery. Secondary outcomes were 30-day mortality and duration of ICU admission (<48 hours *vs* ≥48 hours).

Statistical analyses

Baseline characteristics were presented as mean with standard deviation (SD), median (25^{th} – 75^{th} percentile) or number (percentage), for all patients, and separately for patients who did and did not require RRT after cardiac surgery. Biomarker levels were log-transformed by the natural logarithm to normalize their distributions. For descriptive statistics, logarithmically transformed biomarker levels were back-transformed to the original scale to present geometric means and 95% confidence intervals (CIs). The proportion of missing values was 4.3% for baseline urinary TIMP-2 and IGFBP7, and 7.0% for urinary TIMP-2 and IGFBP7 levels post-surgery. The EuroSCORE could be determined in 99.4% of all patients and a baseline serum creatinine value was available for 99.7% of the entire cohort. There were no missing data on the outcomes RRT, long ICU stay, and 30-day mortality. In the main analyses we included complete cases only.

We used univariate logistic regression to assess the association of pre- and post-surgery biomarker levels, as well as the relative change between pre- and post-surgery levels, and occurrence of RRT, long ICU stay, and 30-day mortality. Discrimination of each biomarker was assessed by the C-statistic. We calculated C-statistics using weighted analyses, to adjust for the oversampling of patients with a short ICU stay, since unweighted analyses in case-control studies may lead to an underestimation of the C-statistic.[12]

We subsequently assessed whether IGFBP7 or TIMP-2 improved the performance of a pre-specified model containing a marker of kidney function, or a general ICU model to predict post-surgery mortality. Both models also included information on procedural complications. First, we assessed whether each biomarker improved a model containing baseline creatinine and duration of surgery. Pre-surgery models included only baseline serum creatinine, whereas post-surgery models also included duration of surgery. Second, we assessed whether both biomarkers improved performance of the EuroSCORE model, regarding primary and secondary outcomes. As a more general model, we used the EuroSCORE model, which combines a variety of dichotomized parameters concerning health status into an additive score. We compared C-statistics of models with and without each biomarker. Since the C-statistic is a relatively insensitive measure for model improvement, we also assessed the category-free net reclassification improvement (cf-NRI) and integrated discrimination improvement (IDI). [13] Briefly, when calculating the cf-NRI, a new model is considered superior

if a higher risk is assigned to an individual with the outcome, and a lower risk to an individual without the outcome, compared with the old model. [14] There are no official benchmarks for the cf-NRI, but values above 0.6 are suggested to indicate strong model improvement, values between 0.2 and 0.6 moderate, and less than 0.2 weak.[15] The IDI represents the difference in discrimination slopes between the old and new models.[16] The discrimination slope is the difference between the mean predicted risk in patients with *vs* without the outcome.[16] For the IDI, there are no cut-offs to determine the magnitude of model improvement. The NRI and IDI do not require weighting, provided that selected controls are a representative sample of the underlying cohort.[12,17]

Finally, we assessed whether the absolute or relative difference between pre- and post-surgery biomarker levels predicted need for RRT. We also investigated the product of both biomarkers, [TIMP-2]·[IGFBP7], which has shown good predictive value in previous publications.[8]

Sensitivity analyses

We repeated the analyses after multiple imputation, assuming data were missing at random. We used 10 imputations and included all relevant baseline variables and the outcome in the model. We derived standard errors of pooled estimates using Rubin's rules.[18] We also repeated the analyses after adjusting urinary biomarkers for urinary creatinine and urine osmolality, to correct for physiological variation in urinary concentration.

We considered two-sided *P*-values <0.05 statistically significant. All analyses were performed using STATA Statistical Software version 14 (Statacorp, Texas, USA) and SPSS 25.0 (IBM Corp., Armonk, NY, USA). We used the *idi* STATA package by Mark Lunt to calculate the NRI and IDI.

RESULTS

Baseline characteristics

Baseline characteristics are presented in Table 1 for all patients, and separately for patients with or without need of RRT. The majority (n=16/22) of all patients who required RRT did so within 3 days post-surgery. For all patients, mean age was 66 years, 67% were men and the proportion of patients with cardiovascular comorbidity was high (Table 1). Eighteen patients died within 30 days. Patients requiring RRT compared with patients not needing RRT, had lower baseline estimated glomerular filtration rate (eGFR), higher APACHE IV score, longer duration of surgery, and had more frequently hypertension and heart failure. Baseline biomarker levels did not correlate with serum creatinine (p >0.3); only

post-surgery TIMP-2 levels weakly correlated with serum creatinine (Pearson correlation 0.13, p=0.04).

Table 1: Baseline characteristics of all 344 elective cardiac surgery patients and stratified according to start of renal replacement therapy (RRT).

	All patients (n=344)	No RRT (n=322)	RRT (n=22)
Age, y	66 ± 11	66 ± 11	69 ± 13
Sex, % men	229 (67)	213 (66)	16 (73)
Current smoker, no. (%)	82 (24)	76 (24)	6 (27)
Body-mass index, kg/m^2	27 ± 4	27 ± 4	28 ± 6
Duration of surgery, min	288 ± 118	311 ± 136	402 ± 131
APACHE IV score[a]	49 ± 16	48 ± 15	97 ± 49
EuroSCORE[b]	5.4 ± 2.7	5.4 ± 2.7	7.3 ± 5.3
Hypertension,[c] no. (%)	163 (47)	155 (48)	15 (68)
BP-lowering drugs,[d] no. (%)	219 (64)	202 (63)	17 (77)
ACE-inhibiting drugs, no. (%)	201 (58)	186 (58)	15 (68)
Lipid-modifying drugs,[d] no. (%)	211 (61)	198 (62)	13 (59)
Diabetes,[e] no. (%)	72 (21)	67 (21)	5 (23)
Heart failure, no. (%)	82 (24)	71 (22)	11 (50)
Serum cystatin C, mg/L	1.04 ± 0.32	1.04 ± 0.30	1.93 ± 1.15
Serum creatinine,[f] µmol/L	81 ± 24	81 ± 23	140 ± 85
$eGFR_{cysC}$, mL/min/1.73m^2	75 ± 24	77 ± 23	41 ± 48
$eGFR_{cr-cysC}$, mL/min/1.73m^2	77 ± 22	80 ± 21	45 ± 44
Urinary protein, g/L	0.15 ± 0.21	0.15 ± 0.21	0.16 ± 0.21
Urinary creatinine, mmol/L	10.6 ± 0.3	10.6 ± 0.3	9.6 ± 1.3
Urinary osmolality, mOsmol/kg	549 ± 10	552 ± 10	504 ± 32

ACE, angiotensin-converting enzyme; BP, blood pressure; cr, creatinine; cysC, cystatin C; eGFR, estimated glomerular filtration rate; EuroSCORE, European System for Cardiac Operative Risk Evaluation.
Data are reported as number of patients (%), mean ± SD or median (25^{th} – 75^{th} percentile).
[a] The APACHE IV score included information on age, temperature, mean arterial pressure, heart rate, respiratory rate, oxygenation, arterial pH, serum sodium and potassium, urine output, serum creatinine, liver function, hematocrit, white blood cell count, Glasgow Coma Scale, and presence of several chronic health conditions.
[b] The EuroSCORE consists of 17 items, including age, sex, chronic pulmonary disease, previous cardiac surgery, serum creatinine, left ventricular dysfunction, and whether a procedure was planned or emergency.[10]
[c] Diagnosis by a physician according to electronic medical records.
[d] Blood pressure-lowering drugs ATC codes C02, C03, C07, C08, and C09. Lipid-modifying drugs ATC code C10AA.
[e] Self-reported diagnosis by a physician, use of glucose-lowering drugs, or hyperglycemia.
[f] To convert the values for creatinine to mg/dL divide by 88.40.

Profile of urinary biomarker levels before and after elective cardiac surgery

Pre-operative mean urinary TIMP-2 levels were comparable in patients who did or did not develop severe AKI necessitating RRT post-surgery However, post-operative TIMP-2 levels were 2.7-fold higher in patients who started RRT (Figure 2, Supplementary Table S1). In contrast, IGFBP7 levels were consistently increased both pre- and post-surgery, in RRT patients compared with non-RRT patients. For the outcome duration of ICU stay ≥48 hours and 30-day mortality, post-surgery TIMP-2 and IGFBP7 levels were about 1.5-fold and 2-fold higher, respectively (Supplementary Table S1).

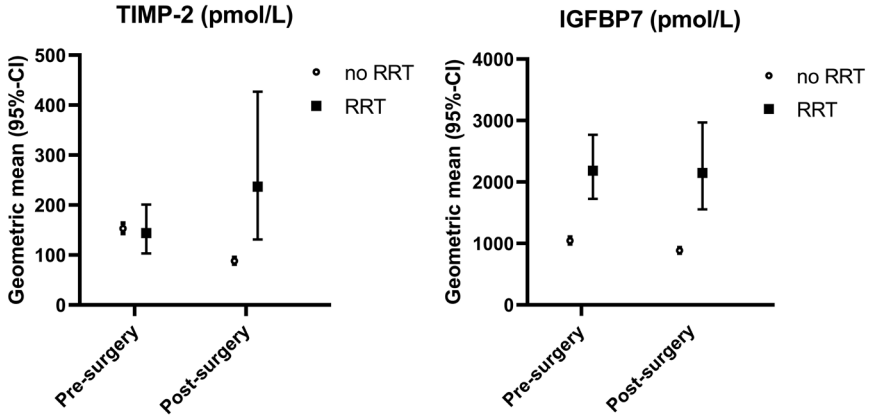

Figure 2: Urinary biomarker concentrations among cardiac patients, pre- and post-surgery according to need of renal replacement therapy. CI, confidence interval; IGFBP7, insulin-like growth factor-binding protein 7; RRT, renal replacement therapy; TIMP-2, tissue inhibitor of metalloprotease-2.

Discriminative value of urinary biomarkers

Pre-surgery TIMP-2 levels did not discriminate between patients who did or did not start RRT post-surgery (Figure 2). Pre-surgery IGFBP7 reasonably discriminated need for RRT, with a C-statistic of 0.77 (95%-CI: 0.69; 0.85). However, baseline serum creatinine performed considerably better, with a C-statistic of 0.85 (0.75; 0.95). Combining serum creatinine with baseline IGFBP7 yielded a C-statistic of 0.92 (0.88; 0.96). Including the duration of surgery, as a proxy for procedural complications, also increased the C-statistic to 0.92 (0.88; 0.97). Despite the high C-statistic of a simple model consisting of baseline serum creatinine and duration of surgery, risk classification significantly improved upon addition of post-surgery IGFBP7 or TIMP-2 levels (Table 2). The

EuroSCORE model plus duration of surgery effectively predicted need for RRT, and improved upon adding either biomarker, especially IGFBP7 (Supplementary Table S2).

Table 2: Discrimination and reclassification performance with 95% CI of urinary biomarkers, change in biomarkers during surgery, and the biomarker product [TIMP-2]•[IGFBP7], regarding risk of renal replacement therapy.

	C-statistic [a] (95% CI)	C-statistic [a] (95% CI)	cf-NRI (95% CI)	IDI (95% CI)
Pre-surgery	Biomarker	+ serum creatinine		
	-	0.85 (0.75; 0.95)**	-	-
TIMP-2	0.45 (0.32; 0.58)	0.85 (0.74; 0.95)**	0.22 (-0.23; 0.64)	-0.002 (-0.008; 0.003)
IGFBP7	0.77 (0.69; 0.85)*	0.92 (0.88; 0.96)**	0.80 (0.37; 1.23)*	0.029 (0.005; 0.052)*
[TIMP-2]•[IGFBP7]	0.58 (0.46; 0.70)	0.90 (0.86; 0.95)**	0.61 (0.18; 1.04)*	0.002 (-0.011; 0.015)
Post-surgery	Biomarker	+ serum creatinine and surgery duration		
	-	0.92 (0.88; 0.97)**	-	-
TIMP-2	0.77 (0.66; 0.88)*	0.93 (0.90; 0.97)**	0.73 (0.29; 1.18)*	0.010 (-0.029; 0.048)
IGFBP7	0.78 (0.69; 0.87)*	0.93 (0.87; 0.98)**	0.81 (0.37; 1.25)*	0.044 (-0.001; 0.089)
TIMP-2 change [b]	0.76 (0.65; 0.87)*	0.92 (0.87; 0.98)**	0.61 (0.16; 1.05)*	0.011 (-0.017; 0.038)
IGFBP7 change [b]	0.55 (0.45; 0.66)	0.92 (0.87; 0.97)**	0.30 (-0.21; 0.81)	0.001 (-0.004; 0.006)
[TIMP-2]•[IGFBP7]	0.80 (0.69; 0.91)*	0.92 (0.88; 0.97)**	0.82 (0.38; 1.26)**	0.033 (-0.016; 0.082)

cf-NRI, category-free net reclassification improvement; CI, confidence interval; IDI, integrated discrimination improvement; IGFBP7, insulin-like growth factor-binding protein 7; TIMP-2, tissue inhibitor of metalloprotease-2.
* p <0.05, ** p <0.001
[a] Analyses were weighted towards the distribution of long *vs* short intensive care unit (ICU) stay of the original cohort.
[b] Change means the absolute difference between pre-surgery and post-surgery levels.

Change in IGFBP7 levels did not predict RRT (Table 2), while changes in TIMP-2 reasonably predicted RRT and improved risk classification of patients when added to a model containing baseline serum creatinine and duration of surgery. In general, C-statistics for the product of both biomarkers [TIMP-2]·[IGFBP7] were comparable to C-statistics for each biomarker individually (Table 2). Pre-surgery [TIMP-2]·[IGFBP7] poorly predicted RRT, with a C-statistic of 0.58 (95% CI: 0.46; 0.70), while post-surgery the C-statistic was 0.80 (0.69; 0.91).

Overall, both biomarkers were poor predictors of long ICU stay. IGFBP7 significantly improved model discrimination when added to a model consisting of baseline serum creatinine and duration of surgery, whereas TIMP-2 did not influence model discrimination (Supplementary Table S3). Similar results were obtained when either biomarker was added to a model consisting of the EuroSCORE and duration of surgery (Supplementary Table S4).

Pre-surgery biomarker levels did not predict 30-day mortality. In contrast, post-surgery levels significantly improved discrimination of a model consisting of serum creatinine or the EuroSCORE, and duration of surgery (Supplementary Tables S5 and S6). The C-statistic for IGFBP7 alone was 0.80 (95% CI: 0.73; 0.87) and was 0.87 (0.79; 0.94) for a model containing serum creatinine, duration of surgery, and post-surgery IGFBP7.

Results after multiple imputation were similar compared to the complete case analyses . Adjusting for urine osmolality did not change the results (Supplementary Table S7).

DISCUSSION

We showed in a cohort of elective cardiac surgery patients that urinary IGBP7 pre- and post-surgery or a change in TIMP-2 levels reasonably predicted the need for RRT with a C- statistic of about 0.80. Interestingly, a simple model consisting of baseline serum creatinine and duration of surgery had very good discriminative power with a C-statistic of 0.92, which was further improved to 0.93 upon addition of post-surgery TIMP-2 or IGFBP7. The product of [TIMP-2]·[IGFBP7] had comparable performance with each biomarker individually. Both biomarkers poorly predicted long ICU stay. Post-surgery levels of TIMP-2 and IGFBP7 had reasonably to good discriminative value regarding 30-day mortality with C-statistics of 0.74 and 0.80, respectively.

To the best of our knowledge, no studies have investigated the potential role of the urinary biomarkers TIMP-2 or IGFBP7 for the prediction of RRT after elective cardiac surgery. Previous research included 50 to 100 patients, and studied the product of both biomarkers, [TIMP-2]·[IGFBP7] and the risk

of AKI stage 2 or 3. However, these studies did not investigate each biomarker separately. Several studies found a good C-statistic for [TIMP-2]·[IGFBP7] 1 day post-surgery of >0.80 regarding any stage of AKI.[19-22] Others reported an area under the curve (AUC) of about 0.5 immediately after surgery or 0.69 at 12 hours post-surgery.[23,24] Bell *et al* found that [TIMP-2]·[IGFBP7] did not predict AKI within 48 hours after ICU admission in general ICU patients. Additionally, they showed that biomarker levels were significantly affected by comorbidities such as diabetes, challenging the robustness of these biomarkers.[25] A recent meta-analysis on the predictive value of urinary [TIMP-2]·[IGFBP7] concluded that it is an effective test for cardiac surgery-associated AKI, with a pooled C-statistic of 0.83.[26] However, all studies were pooled regardless of the timing of biomarker assessment. In contrast, in the present study we studied whether biomarker levels pre-surgery and directly post-surgery would be valuable predictors of AKI or RRT in an early stage. We found that both biomarkers directly post-surgery may be of value in predicting RRT. However, prediction of the need for RRT by a model containing only baseline serum creatinine and duration of surgery was already very high, leaving little room for improvement by additional biomarkers.

Data on the predictive value of both biomarkers with regard to mortality is scarce. Koyner *et al* found that [TIMP-2]·[IGFBP7] did not improve the C-statistic of 0.70 of a clinical model predicting a composite outcome of death or dialysis within 9 months in critically ill patients. However, there was some improvement of both reclassification indices NRI and IDI.[27] Others showed in 98 critically ill patients that TIMP-2 predicted stage 3 AKI with a C-statistic of 0.80, and a C-statistic of 0.83 for 7-day mortality.[28] Importantly, critically ill patients are not comparable to elective cardiac surgery patients included in the present study. Critically ill patients consist of a heterogeneous population, admitted to the ICU for a variety of medical reasons, such as sepsis, coma, and respiratory insufficiency, and include emergency admissions.

We found two-fold higher pre-surgery IGFBP7 levels in elective cardiac surgery patients who started RRT post-surgery versus patients not needing RRT. In contrast, pre-surgery TIMP-2 levels were comparable in patients who did or did not need RRT, but substantially increased post-surgery in patients who needed RRT. These results suggest that IGFBP7, which is mainly a proximal tubular marker, may be chronically elevated in part of the cardiac patients, while TIMP-2 increases only upon severe tubular damage. TIMP-2 levels especially increased during surgery in patients needing RRT. In line with these observations, in a cohort of kidney transplant patients, only TIMP-2 was a good predictor of delayed graft function. In contrast, IGFBP7 was elevated in most patients, which means discriminative value was poor.[29] Finally, we performed

an additional analysis after adjusting urinary biomarker levels for urinary osmolality, since osmolality may influence biomarker levels.[30] We showed that results were comparable with or without adjustment for urinary osmolality.

This study had several limitations. First, because serum and urinary output measurements were recorded as part of routine care, the number of missing values for these variables was relatively high. Therefore, information on development of milder stages of AKI, e.g. stage 1 or 2, was not available. Additionally, sampling was terminated after patients left the ICU. We could therefore not continue monitoring biomarker levels after patients had left the ICU. Second, there were relatively few events of RRT or 30-day mortality, which prevented the incorporation of additional variables in our multivariable models. Nonetheless, discrimination was high using simple models, especially when predicting RRT.

The main strength of this study is the large homogenous sample of elective cardiac surgery patients. Second, we investigated different relevant endpoints, and additionally to standard methods, we used the newer indices for risk reclassification, NRI and IDI, as further measures of model improvement. Importantly, though both biomarkers measured post-surgery have good discrimination with regards to need for RRT, a biomarker with high discriminative value when measured pre-surgery would be much more clinically relevant. A predicted high risk for need for RRT or mortality post-surgery may aid in deciding which patients should be monitored more closely after surgery, but it has no implication on whether surgery should be performed in the first place.

In conclusion, we found that both TIMP-2 and IGFBP7 improved discrimination and risk classification of patients regarding RRT after elective cardiac surgery. Prediction of 30-day mortality was reasonable for both biomarkers, but was poor for long ICU stay. We found no evidence that the product [TIMP-2]·[IGFBP7] performed better than each biomarker alone. Discrimination regarding RRT after cardiac surgery was already very high using clinical variables such as baseline serum creatinine and duration of surgery. Nonetheless, in elective cardiac surgery patients, urinary TIMP-2 and IGFBP7 improved prediction of RRT post-surgery.

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Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests

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This study did not receive external funding.

AUTHORS' CONTRIBUTIONS

Research idea and study design: JF, AS, KE, EH, CC, DS; data acquisition: AS, RR, FR, JD, MS, CC, KE; data analysis/interpretation: JF, AS, KE, EH, DS, FR, RR, MS, CC; statistical analysis: KE, EH; supervision and mentorship: JF, EH, AS, CC. All authors read and approved the final manuscript.

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SUPPLEMENTARY DATA

Table S1: Urinary biomarker level pre- and post-surgery in cardiac surgery patients according to length of stay at the ICU (≥48 hours *vs* <48 hours) and 30-day mortality.

	TIMP-2 (pmol/L) Geometric mean (95% CI)		IGFBP7 (pmol/L) Geometric mean (95% CI)	
	Pre-surgery	Post-surgery	Pre-surgery	Post-surgery
ICU stay ≥48 hours				
No	154 (143; 167)	85 (78; 92)	963 (897; 1034)	821 (766; 878)
Yes	146 (128; 167)	117 (97; 142)	1525 (1349; 1725)	1284 (1119; 1473)
Ratio	0.9 (0.8; 1.1)	1.4 (1.1; 1.7)	1.6 (1.4; 1.8)	1.6 (1.3; 1.8)
p for ratio [a]	0.5	0.001	<0.001	<0.001
30-day mortality				
No	154 (144 (165)	89 (82; 96)	1034 (998; 1134)	889 (475; 1663)
Yes	117 (75; 185)	179 (112; 288)	1300 (864; 1956)	1821 (1262; 2627)
Ratio	0.8 (0.5; 1.1)	2.0 (1.2; 3.5)	1.2 (0.8; 1.8)	2.0 (1.3; 3.2)
p for ratio [a]	0.2	0.02	0.3	0.001

CI, confidence interval; ICU, intensive care unit; IGFBP7, insulin-like growth factor-binding protein 7; TIMP-2, tissue inhibitor of metalloprotease-2.
[a] Ratio means relative difference in biomarker levels between both groups.

Table S2: Discrimination and reclassification performance of urinary biomarkers regarding risk of renal replacement therapy.

	C-statistic [a] (95% CI)	cf-NRI (95% CI)	IDI (95% CI)
Pre-surgery			
TIMP-2	0.45 (0.32 (0.58)	-	-
IGFBP7	0.77 (0.69; 0.85)	-	-
EuroSCORE	0.65 (0.55; 0.75)	-	-
EuroSCORE + TIMP-2	0.65 (0.55; 0.76)	0.32 (-0.11; 0.75)	0.0004 (-0.0008; 0.0016)
EuroSCORE + IGFBP7	0.80 (0.73; 0.88)[b]	0.71 (0.28; 1.14)*	0.018 (0.002; 0.033)*
Post-surgery			
TIMP-2	0.77 (0.66; 0.88)	-	-
IGFBP7	0.78 (0.68; 0.89)	-	-
EuroSCORE + surgery duration	0.79 (0.71; 0.88)	-	-
EuroSCORE + surgery duration + TIMP-2	0.81 (0.69; 0.92)	0.58 (0.12; 1.03)*	0.089 (0.022; 0.156)*
EuroSCORE + surgery duration + IGFBP7	0.84 (0.74; 0.94)	1.02 (0.57; 1.47)**	0.098 (0.051; 0.144)*

cf-NRI, category-free net reclassification improvement; EuroSCORE, European System for Cardiac Operative Risk Evaluation; IDI, integrated discrimination improvement; IGFBP7, insulin-like growth factor-binding protein 7; TIMP-2, tissue inhibitor of metalloprotease-2
* $p < 0.05$, ** $p < 0.001$
[a] Analyses were weighted towards the distribution of long *vs* short ICU stay of the original cohort.
[b] Statistically significant improvement of C-statistic, compared to a model containing the EuroSCORE alone.

Table S3: Discrimination and reclassification of urinary biomarkers regarding risk of long ICU stay, and improvement of a renal model.

	C-statistic [a] (95% CI)	cf-NRI (95% CI)	IDI (95% CI)
Pre-surgery			
TIMP-2	0.47 (0.43; 0.52)	-	-
IGFBP7	0.66 (0.61; 0.70)	-	-
Serum creatinine[b]	0.61 (0.56; 0.66)	-	-
Serum creatinine[b] + TIMP-2	0.61 (0.56; 0.66)	0.07 (-0.15; 0.28)	0.002 (-0.003; 0.008)
Serum creatinine[b] + IGFBP7	0.70 (0.65; 0.74)[c]	0.43 (0.21; 0.65)**	0.059 (0.034; 0.084)**
Post-surgery			
TIMP-2	0.60 (0.55; 0.66)	-	-
IGFBP7	0.65 (0.60; 0.70)	-	-
Serum creatinine[b] + surgery duration	0.72 (0.67; 0.76)	-	-
Serum creatinine[b] + surgery duration + TIMP-2	0.72 (0.67; 0.77)	0.15 (-0.07; 0.37)	0.006 (-0.002; 0.014)
Serum creatinine[b] + surgery duration + IGFBP7	0.74 (0.69; 0.78)	0.29 (0.07; 0.51)*	0.036 (0.015; 0.057)**

cf-NRI, category-free net reclassification improvement; CI, confidence interval; IDI, integrated discrimination improvement; ICU, intensive care unit; IGFBP7, insulin-like growth factor-binding protein 7; TIMP-2, tissue inhibitor of metalloprotease-2
* p <0.05, ** p <0.001
[a] Analyses were weighted towards the distribution of long *vs* short ICU stay of the original cohort.
[b] Serum creatinine at baseline, before surgery.
[c] Statistically significant improvement of C-statistic, compared to the European System for Cardiac Operative Risk Evaluation (EuroSCORE) model.

Table S4: Discrimination and reclassification of urinary biomarkers regarding risk of long ICU stay, and improvement of the EuroSCORE model.

	C-statistic [a] (95% CI)	cf-NRI (95% CI)	IDI (95% CI)
Pre-surgery			
TIMP-2	0.47 (0.43; 0.52)	-	-
IGFBP7	0.66 (0.61; 0.70)	-	-
EuroSCORE	0.63 (0.58; 0.68)	-	-
EuroSCORE + TIMP-2	0.64 (0.59; 0.69)	0.12 (-0.09; 0.34)	0.005 (-0.003; 0.013)
EuroSCORE + IGFBP7	0.69 (0.65; 0.73)	0.41 (0.19; 0.63)**	0.049 (0.026; 0.072)**
Post-surgery			
TIMP-2	0.60 (0.55; 0.66)	-	-
IGFBP7	0.65 (0.60; 0.70)	-	-
EuroSCORE + surgery duration	0.70 (0.66; 0.75)	-	-
EuroSCORE + surgery duration + TIMP-2	0.70 (0.65; 0.75)	0.17 (-0.05; 0.39)	0.006 (-0.002; 0.014)
EuroSCORE + surgery duration + IGFBP7	0.73 (0.68; 0.78)	0.24 (0.02; 0.46)*	0.042 (0.020; 0.064)**

cf-NRI, category-free net reclassification improvement; CI, confidence interval; EuroSCORE, European System for Cardiac Operative Risk Evaluation; IDI, integrated discrimination improvement; IGFBP7, insulin-like growth factor-binding protein 7; TIMP-2, tissue inhibitor of metalloprotease-2
* p <0.05, ** p <0.001
[a] Analyses were weighted towards the distribution of long (≥48 hours) *vs* short (<48 hours) intensive care unit (ICU) stay of the original cohort.

Table S5: Discrimination and reclassification of urinary biomarkers regarding the outcome 30-day mortality, and improvement of a renal model.

	C-statistic [a] (95% CI)	cf-NRI (95% CI)	IDI (95% CI)
Pre-surgery			
TIMP-2	0.41 (0.26; 0.56)	-	-
IGFBP7	0.58 (0.44; 0.72)	-	-
Serum creatinine[b]	0.63 (0.50; 0.75)	-	-
Serum creatinine[b] + TIMP-2	0.66 (0.53; 0.78)	-0.05 (-0.52; 0.42)	-0.0003 (-0.001; 0.001)
Serum creatinine[b] + IGFBP7	0.63 (0.48; 0.78)	0.37 (-0.10; 0.84)	0.005 (-0.003; 0.013)
Post-surgery			
TIMP-2	0.74 (0.63; 0.85)	-	-
IGFBP7	0.80 (0.73; 0.87)	-	-
Serum creatinine[b] + surgery duration	0.75 (0.64; 0.86)	-	-
Serum creatinine[b] + surgery duration + TIMP-2	0.80 (0.70; 0.91)	0.43 (-0.12; 0.98)	0.004 (-0.008; 0.016)
Serum creatinine[b] + surgery duration + IGFBP7	0.87 (0.79; 0.94) [c]	0.63 (0.08; 1.18)*	0.012 (-0.015; 0.039)

cf-NRI, category-free net reclassification improvement; CI, confidence interval; IDI, integrated discrimination improvement; IGFBP7, insulin-like growth factor-binding protein 7; TIMP-2, tissue inhibitor of metalloprotease-2
* p <0.05
[a] Analyses were weighted towards the distribution of long *vs* short ICU stay of the original cohort.
[b] Serum creatinine at baseline, before surgery.
[c] Statistically significant improvement of C-statistic, compared to a model including the EuroSCORE + surgery duration.

Table S6: Discrimination and reclassification of urinary biomarkers regarding the outcome 30-day mortality, and improvement of the EuroSCORE model.

	C-statistic [a] (95% CI)	cf-NRI (95% CI)	IDI (95% CI)
Pre-surgery			
TIMP-2	0.41 (0.26; 0.56)	-	-
IGFBP7	0.58 (0.44; 0.72)	-	-
EuroSCORE	0.68 (0.58; 0.77)	-	-
EuroSCORE + TIMP-2	0.70 (0.59; 0.82)	0.22 (-0.25; 0.69)	0.0001 (-0.001; 0.001)
EuroSCORE + IGFBP7	0.69 (0.61; 0.78)	0.17 (-0.30; 0.64)	0.001 (-0.006; 0.008)
Post-surgery			
TIMP-2	0.74 (0.63; 0.85)	-	-
IGFBP7	0.80 (0.73; 0.87)	-	-
EuroSCORE + surgery duration	0.77 (0.70; 0.84)	-	-
EuroSCORE + surgery duration + TIMP-2	0.80 (0.69; 0.91)	0.76 (0.21;1.31)*	0.018 (0.003; 0.033)*
EuroSCORE + surgery duration + IGFBP7	0.85 (0.79; 0.92) [b]	0.81 (0.26; 1.36)*	0.019 (0.004; 0.034)*

cf-NRI, category-free net reclassification improvement; CI, confidence interval; EuroSCORE, European System for Cardiac Operative Risk Evaluation; IDI, integrated discrimination improvement; IGFBP7, insulin-like growth factor-binding protein 7; TIMP-2, tissue inhibitor of metalloprotease-2

* $p < 0.05$

[a] Analyses were weighted towards the distribution of long *vs* short intensive care unit (ICU) stay of the original cohort.

[b] Statistically significant improvement of C-statistic, compared to a model including the EuroSCORE + surgery duration.

Table S7: Discrimination and reclassification of osmolality-adjusted urinary biomarkers regarding the outcome renal replacement therapy (RRT).

	C-statistic [a] (95% CI)	cf-NRI (95% CI)	IDI (95% CI)
Pre-surgery			
TIMP-2	0.60 (0.48; 0.72)	-	-
IGFBP7	0.67 (0.56; 0.78)	-	-
Serum creatinine	0.85 (0.74; 0.96)	-	-
Serum creatinine + TIMP-2	0.84 (0.73; 0.96)	-0.24 (-0.69; 0.21)	-0.000 (-0.0004; 0.0004)
Serum creatinine + IGFBP7	0.85 (0.74; 0.96)	0.22 (-0.23; 0.67)	0.003 (-0.001; 0.007)
Post-surgery			
TIMP-2	0.75 (0.63; 0.87)	-	-
IGFBP7	0.75 (0.64; 0.86)	-	-
Serum creatinine + surgery duration	0.92 (0.88; 0.97)	-	-
Serum creatinine + surgery duration + TIMP-2	0.93 (0.89; 0.96)	0.48 (0.03; 0.93)*	-0.0004 (-0.014; 0.013)
Serum creatinine + surgery duration + IGFBP7	0.92 (0.87; 0.96)	0.41 (-0.04; 0.86)	0.003 (-0.004; 0.010)

cf-NRI, category-free net reclassification improvement; IDI, integrated discrimination improvement; IGFBP7, insulin-like growth factor-binding protein 7; TIMP-2, tissue inhibitor of metalloprotease-2
* p <0.05
[a] Analyses were weighted towards the distribution of long *vs* short intensive care unit (ICU) stay of the original cohort.

Chapter 8 -

Low birth weight and kidney function in middle-aged men and women: The Netherlands Epidemiology of Obesity Study

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ABSTRACT

Rationale and objective: Chronic kidney disease (CKD), defined as estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m^2, is a risk factor for cardiovascular morbidity and mortality. Little is known about low birth weight and the risk of CKD in middle-aged adults in the general population. Therefore, we investigated the association between birth weight and eGFR in a Dutch cohort of middle-aged men and women. We also studied the causal relation between birth weight and eGFR using genetic variants associated with birth weight as instrument.

Study design: observational study.

Setting and participants: 6,671 participants of the Netherlands Epidemiology of Obesity (NEO) study. Validation study with data on 133,814 participants of the CKDgen consortium.

Exposure: Birth weight was both self-reported, and based on an instrument, including 59 birth weight-associated genetic variants, derived from an independent data source.

Outcome: eGFR at the age of 45-65 years.

Analytical approach: We assessed the association between self-reported birth weight and eGFR in the NEO-study by multivariable linear regression, adjusted for age, sex, education, smoking, and alcohol use. The effect of the instrument for genetic low birth weight on eGFR was estimated by two separate two-sample Mendelian randomization analyses: with individual data from the NEO cohort and summary data from the CKDgen consortium.

Results: At baseline, mean (SD) eGFR was 86 (12.4) mL/min/1.73m^2. After multivariable adjustment, self-reported birth weight was not associated with kidney function at middle age. Two-sample Mendelian randomization analysis showed that in the NEO cohort each 500 gram genetically decreased birth weight was related to a 3.7 (95%-CI: 0.5; 6.9) mL/min/1.73m^2 lower kidney function at the age of 45-65 years. However, using CKDgen summary level data, showed no significant relation between birth weight and eGFR in middle-aged adults.

Limitations: Birth weight was self-reported.

Conclusion: Each 500 gram genetic lower birth weight was related with 3.7 ml/min/1.73m^2 lower kidney function at middle age. However, we could not validate this result in the CKDgen cohort.

INTRODUCTION

In Europeans ≥45 years, the prevalence of CKD, defined as estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m^2, is high, at 11%.[1–3] CKD increases the risk of cardiovascular morbidity, mortality and end-stage renal disease (ESRD).[4] Classic cardiovascular risk factors, such as diabetes, smoking and hypertension can only explain part of the risk of CKD in adults. Therefore, identification of novel risk factors of CKD is important for targeted prevention of kidney function decline.

A low number of glomeruli at birth may predispose for CKD in adults. The number of glomeruli varies substantially across individuals, ranging from 300,000-2,000,000 per kidney.[5] Birth weight is a strong determinant for glomerular mass: each additional kg birth weight is associated with about 250,000 extra glomeruli per kidney.[5, 6] Human autopsy studies showed that a lower number of glomeruli was associated with a larger nephron volume, which suggests hyperfiltration.[6–8] Brenner hypothesized that adults with a congenital reduction in the number of glomeruli have a greater likelihood of developing hypertension and subsequent kidney failure.[9, 10] The mechanistic explanation for this phenomenon is that compensatory hyperfiltration by the remaining glomeruli results in accelerated kidney function decline. In addition, lower birth weight has been associated with increased insulin resistance, higher fasting insulin concentrations and increased incidence of type 2 diabetes mellitus.[11]

A recent meta-analysis, including almost 50,000 individuals from 31 studies, showed that low birth weight was associated with a 70% increased risk of CKD in adult life.[12] However, the majority of included studies consisted of highly selected samples of the population, consisting of subjects with diabetes, Pima Indians, or Aboriginals. It cannot be ruled out, that in the positive studies other factors caused both low birth weight and impaired kidney function later in life.

Since it is not known whether low birth weight causes lower kidney function in adults, we studied this relation from three perspectives. First, we examined the association between low birth weight and kidney function in a middle-aged cohort of the general Dutch population: the Netherlands Epidemiology of Obesity (NEO) study. Second, we performed a Mendelian randomization analysis in the NEO study, using a genetic risk score for low birth weight as an instrument in a causal analysis.[13] Finally, we validated the results from this Mendelian randomization analysis using summary level data of 133,814 individuals.[14, 15]

METHODS

Study design and participants

The Netherlands Epidemiology of Obesity (NEO) study is a population-based, prospective cohort study designed to investigate pathways that lead to common disorders. The NEO study included 6,671 individuals aged 45-65 years, with an oversampling of overweight or obese individuals. Men and women aged 45-65 years with a self-reported body mass index (BMI) ≥27 kg/m^2 living in the greater area of Leiden (in the West of the Netherlands) were eligible to participate. In addition, all inhabitants aged 45-65 years from one municipality (Leiderdorp) were invited to participate irrespective of their BMI, allowing a reference distribution of BMI. In total, 6,671 participants entered the study, of whom 5,000 with a BMI of 27 kg/m^2 or higher (Supplementary Figure S1). The study design and population are described in detail elsewhere.[16] The Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the design of the study (approval number P08.109). All participants gave written informed consent.

For the validation study we used data from 133,814 European participants of the CKDgen consortium. The CKDgen consortium includes data from 70 population-based studies, with a mean age between 50-60 years and a prevalence of CKD of 5-20%, defined as an eGFR <60 mL/min/1.73m^2.[14] This study was reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[17]

Data collection

Participants were invited to a baseline visit at the NEO study centre of the LUMC after an overnight fast. At the baseline visit participants were physically examined, blood samples were drawn, medication was registered, and questionnaires regarding demographic, lifestyle, and clinical information, including birth weight, were obtained.[16] Patients were asked which of the following four broad categories of birth weight was applicable: <2.5, 2.5 to <3.0, 3.0 to <4.0, or ≥4.0 kg. We defined low birth weight as a birth weight <2.5 kg, according to the World Health Organization.[18]

Kidney function assessment

At baseline, serum creatinine was measured from fasting blood samples, by the Jaffé kinetic compensated method, or by the enzymatic method (isotope dilution mass spectrometry reference measurement procedure calibrated against standard reference material).[16] Serum Jaffé results were corrected with a fixed compensation factor of -26 µmol/L to compensate for assay non-specificity.

Creatinine-based glomerular filtration rate (eGFR) was estimated using the 2012 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, taking into account age, sex and race.[19] Urinary albumin was measured from spot morning urine samples. In men and women, moderately increased albuminuria was defined as 2.5-25 and 3.5-35 mg/mmol creatinine, and severely increased albuminuria as >25 and >35 mg/mmol creatinine, respectively.

Genetic instrument for birth weight

Genotyping was performed in participants of European ancestry only, using the Illumina HumanCoreExome-24 BeadChip (Illumina Inc., San Diego, California, USA). Genotypes were imputed to the 1000 Genome Project reference panel (v3 2011) using IMPUTE (v2.2) software.[20, 21] We excluded participants with poor genotype data (n=927): sample call rate <98%, sex mismatch, heterozygosity rate not within 3 SD of mean heterozygosity rate, duplicate samples, concordance between samples >0.25, or when participants differed based on the first two principal components (±3.5 SD).

In Mendelian randomization, genetic variants are proposed as instruments to estimate the causal effect of a risk factor (referred to here as an exposure) on an outcome, using observational data.[22] Genetic variants are assumed to be randomly distributed and become fixed at conception, mimicking the distribution of exposure in a randomized trial. Mendelian randomization thus bypasses the main limitation of observational studies: confounding and reverse causality. An instrument must meet the following assumptions: associated with the exposure of interest, only affect the outcome through the exposure (absence of horizontal pleiotropy), and not share any causes with the outcome and as such is independent of confounding factors (Figure 1A).[23] We additionally assume that the assumption of monotonicity holds, under which the causal estimate represents the average causal effect in the genetic "compliers".[24] In case of a continuous exposure, such as birth weight, compliers are those individuals in whom a higher value of the genetic instrument can only increase birth weight, or leave it constant.[24]

We used as instruments 59 autosomal genetic variants (single-nucleotide polymorphisms [SNPs]) reaching genome wide significance in European or trans-ancestry data in a recent genome-wide association study (GWAS) (Supplementary Table S1).[13] In total, these 59 SNPs explained approximately 2% of the birth weight variance. In the NEO-study, we calculated for each participant a weighted risk score by adding up for each individual SNP the number of coding alleles multiplied by their absolute effect on birth weight, based on European ancestry data reported by Horikoshi *et al.* (Figure 1B). As the weights were equal to the expected association of each variant with birth

weight in SDs, a 1-unit increase in genetic risk score corresponded to a 1-SD increase in genetically determined birth weight. which equals 500 gram of birth weight.[13, 25] For the CKDgen data, summary effects for each individual SNP were pooled into a causal estimate.

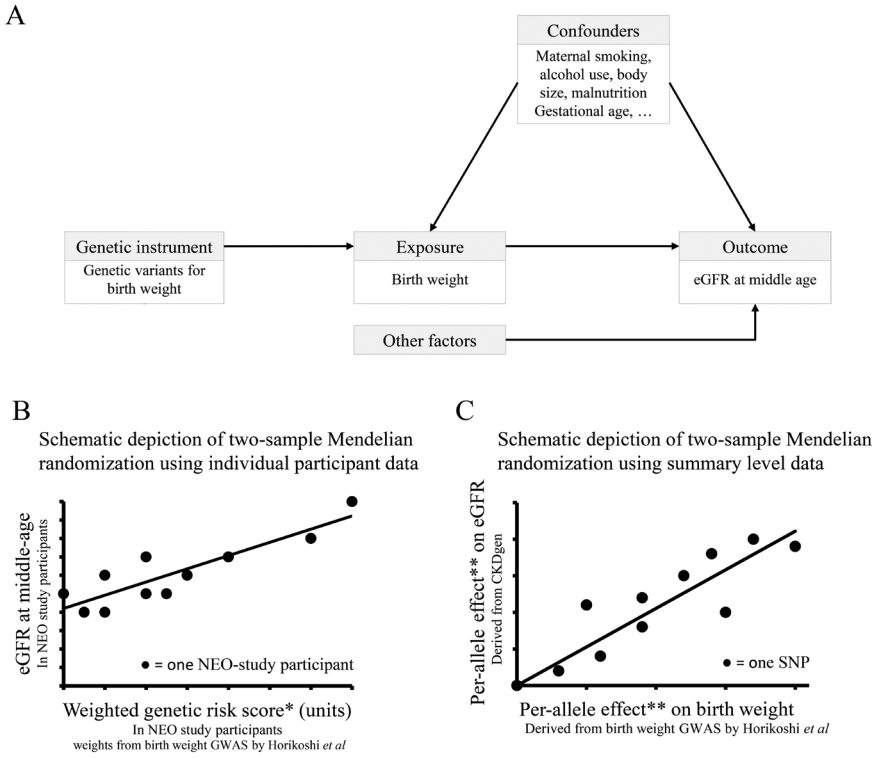

Figure 1: Graphical representation of Mendelian randomization assumptions (A), and schematic depiction of two-sample Mendelian randomization analyses using individual participant data (B) or summary level data (C). A) Basic scheme of the three assumptions of a genetic instrument: associated with the exposure of interest, associated with the outcome only through its association with the exposure and not via other factors, and independent of confounding factors. B) In the NEO cohort we calculated for each participant a weighted genetic risk score, with weights derived from the birth weight GWAS by Horikoshi *et al.*, and used linear regression to investigate the relation of the genetic risk score with eGFR at middle age. The relation is represented by the slope of the regression line. C) In case of two-sample Mendelian randomization using summary level data, for each SNP the per-allele effect on birth weight is contrasted to the per allele effect on eGFR. Both effects were derived from two different GWAS studies. The final causal estimate is represented by the slope of the regression line through all SNPs. *The weighted genetic risk score for every participated was calculated by summing up for each SNP the effect on birth weight multiplied by the number of risk alleles. ** Per-allele effects refer to the regression coefficients from univariable linear regression of the outcome of interest (eGFR) or birth weight, for each SNP.

Statistical analyses

All analyses involving NEO study participants were weighted towards the BMI distribution of the general population, to adjust for the oversampling of individuals with a BMI ≥27 kg/m^2.[26] The weighing procedure is described in detail in Supplementary Figure S2. Baseline characteristics were presented as mean (SD), median (25th - 75th percentile) or percentage, for all participants and across birth weight strata. Assuming missingness was at random, we used multiple imputation for the main analyses. Multiple imputation generally results in less bias than analyzing complete cases only.[27] Missing values were imputed for birth weight (36%), education (1.0%), eGFR (0.7%), urinary albumin (0.4%), ethnicity (0.2%), smoking (0.1%), and alcohol use (<0.1%). We used 10 imputations, including all relevant variables and the outcome into the model. Standard errors of pooled estimates were derived using Rubin's rules.[28] As sensitivity analysis we performed a complete case analysis.

We performed linear regression to examine the relation between self-reported birth weight and eGFR or urinary albumin-to-creatinine ratio (UACR). Logistic regression was used to examine the relation between birth weight and risk of CKD stage 3 (eGFR <60 mL/min/1.73m^2) or albuminuria. Analyses were adjusted for age and sex (model 1). In model 2, we adjusted in addition to model 1, for ethnicity and level of education (high *vs* low). In model 3, we adjusted in addition to model 2, for smoking (current, former, or never) and alcohol consumption (g/day). Finally, we repeated all analyses restricted to Caucasian individuals.

In addition, we conducted two separate two-sample Mendelian randomization analyses, using individual participant data and summary level data. In two-sample Mendelian randomization, the associations between instrument-exposure and instrument-outcome are derived from two different populations or data sources.[15] First, we performed a two-sample Mendelian randomization analysis using individual participant data from the NEO-study (Figure 1B). In this analysis the instrument data were derived from Horikoshi *et al.* and the outcome data from the NEO-study. We used ordinal logistic regression taking birth weight as outcome, to verify the validity of the genetic risk score as instrument for birth weight. We compared age, sex, educational level, diabetes, and obesity across quartiles of the genetic risk score. Subsequently, linear regression was used to quantify the effect of the genetic risk score for birth weight on eGFR at middle age, adjusted for age, sex, and the four most prominent principal components of ancestry.

Second, we performed a two-sample Mendelian randomization analysis using summary level data from the CKDgen consortium. In this analysis instrument-exposure data were derived from Horikoshi *et al.* and the

instrument-outcome data were derived from the CKDgen consortium (Figure 1C).[13, 14] A major advantage of two-sample Mendelian randomization using summary statistics is the increased power. In case of missing SNPs, LD proxies were used ($R^2 > 0.8$) when available, using the 1000 Genomes European sample data in SNAP Proxy Search (Supplementary Table S1 and S2).[29] The presence of LD between SNPs was excluded (n=3) using a threshold of $R^2 > 0.001$). Ultimately, 45 SNPs, including proxies, were available in the CKDgen data. The median (25th – 75th percentile) F-statistic for all 59 SNPs was 35.5 (30.9 – 44.4) and for the 45 SNPs in CKDgen was 33.2 (30.8 – 43.6). Instruments with an F-statistic >10 are generally assumed sufficiently strong to avoid weak instrument bias.[30] The pooled causal estimate in summary level analyses was calculated by regressing the SNP-eGFR effect derived from the CKDgen data on the SNP-birth weight effect derived from Horikoshi *et al.*, weighted by the precision of the SNP-eGFR effect, and with the intercept constrained to zero (Figure 1C).[13, 14] The pooled causal estimate represents the effect of a 1-SD (about 500 gram) increment of genetically increased birth weight on log-transformed eGFR. The IVW method assumes zero horizontal pleiotropy and uses weights that assume no measurement error for the association between SNPs and birth weight.[31, 32]

In addition, we performed several sensitivity analyses. First, we visually examined directional horizontal pleiotropy by leave-one-out and funnel plot analyses. Second, we performed MR-Pleiotropy RESidual Sum and Outlier (MR-PRESSO) analyses, which tests for directional horizontal pleiotropy (MR-PRESSO global test) and detects and corrects for outliers.[33] Third, MR-Egger intercept test was performed, which allows the intercept to deviate from zero to indicate pleiotropy.[32] The intercept from the MR-Egger test can be interpreted as the average pleiotropic effect of all SNPs.[34] The slope of the MR-Egger regression analysis represents the pleiotropy-corrected causal effect. Fourth, we used the weighted median and weighted mode methods. Both methods are less sensitive to outliers, compared to mean-based approaches such as the IVW and MR-Egger method. The weighted median method provides consistent estimates, regardless of horizontal pleiotropy, if at least 50% of the information comes from valid instruments.[35] The weighted mode estimator requires that the most common causal effect estimate comes from valid instruments, even if the majority of instruments is invalid.[36] Finally, a GWAS may not only tag fetal genes associated with birth weight, but also maternal genes, which may influence birth weight through effects on the intrauterine environment. To reduce potential maternal effects of birth weight SNPs, we repeated the analyses excluding SNPs where the maternal effects were strongly associated with birth weight, as reported by Horikoshi *et al.* As threshold we used a Bonferroni corrected p-value of 0.0011 for the maternal association between

each SNP and birth weight, based on 45 SNPs. Analyses in the NEO-study were performed using STATA Statistical Software version 14 (Statacorp, Texas, USA). Two-sample Mendelian randomization analyses using summary level data were performed in R version 3.4.3 (R Foundation for Statistical Computation, Vienna, Austria) using the *TwoSampleMR* and *MR-PRESSO* packages.[33, 37]

RESULTS

Baseline characteristics

Baseline data of all participants and according to four categories of birth weight are presented in Table 1. Mean (SD) eGFR of all participants was 86.2 (12.4) mL/min/1.73m^2. The prevalence of moderately and severely increased albuminuria, and CKD was 2.2%, 0.8%, and 2.2%, respectively. Participants with lower birth weight were more often female, had a lower level of education, had more comorbid conditions, and used more medication. Participants with low birth weight were less likely Caucasian. About 3.2% of all participants with birth weight <2.5 kg were from East-Asian origin. Other ethnic backgrounds were equally distributed across categories of birth weight.

Table 1: Baseline characteristics of 6,671 middle-aged participants of the Netherlands Epidemiology of Obesity study.

		Birth weight (kg)			
	Total population n=6,671	<2.5 (11%)	2.5 to <3.0 (25%)	3.0 to <4.0 (49%)	≥4.0 (15%)
Demographic/anthropometric					
Age (y)	55.7 (6.0)	55.8 (6.1)	55.9 (5.5)	54.9 (6.1)	55.0 (6.4)
Sex (% men)	43.6	29.2	35.2	40.9	52.3
Ethnicity (% Caucasian)	94.9	92.6	96.5	97.7	97.5
Education level (% high)	45.9	38.2	40.5	50.5	51.1
Body-mass index (kg/m^2)	26.3 (4.5)	26.0 (4.4)	25.7 (4.3)	26.1 (4.3)	26.8 (5.4)
Waist circumference (cm)					
Men	98.4 (11.4)	97.1 (9.7)	96.2 (11.4)	97.3 (10.9)	99.9 (13.3)
Women	87.4 (12.6)	87.1 (12.7)	85.6 (12.5)	86.8 (12.3)	86.9 (12.9)
Total body fat (%)					
Men	25.0 (6.1)	24.9 (5.1	24.2 (6.2)	24.5 (5.8)	25.3 (6.8)
Women	36.9 (6.4)	36.3 (6.7)	35.7 (6.9)	36.8 (6.3)	37.0 (7.0)
Current smoking (%)	16.0	15.2	15.6	15.0	16.3
Alcohol intake (g/d)	14.7 (16.3)	11.5 (12.4)	14.4 (16.1)	15.0 (15.6)	14.2 (17.1)
Comorbidity (%)					
Diabetes[a]	5.7	6.5	4.2	4.8	4.2
Heart failure	0.5	1.3	0.4	0.4	0.2
Hypertension	34.0	39.1	36.8	29.6	33.0
Hypercholesterolemia	13.2	16.2	13.9	11.5	9.8
Chronic kidney disease[b]	2.2	2.0	2.3	1.8	1.4
Medication use (%)					
BP-lowering drugs	23.5	28.0	23.1	21.2	19.3
RAS-blockers	13.9	17.8	12.3	12.3	10.9

Table 1: Continued

	Total population n=6,671	Birth weight (kg)			
		<2.5 (11%)	2.5 to <3.0 (25%)	3.0 to <4.0 (49%)	≥4.0 (15%)
Glucose-lowering drugs	2.8	3.3	2.2	2.1	1.7
Statins	10.5	13.5	9.8	8.5	7.0
Laboratory measurements					
Fasting glucose[c] (mg/dL)	99 (18)	99 (16)	97 (14)	97 (18)	97 (16)
Total cholesterol[d] (mg/dL)	220 (43)	220 (39)	224 (39)	220 (39)	217 (43)
HDL cholesterol[d] (mg/dL)	62 (19)	62 (15)	62 (15)	62 (15)	62 (23)
LDL cholesterol[d] (mg/dL)	135 (39)	139 (39)	139 (35)	135 (35)	135 (43)
Serum creatinine[e] (mg/dL)	0.87 (0.16)	0.84 (0.15)	0.85 (0.15)	0.86 (0.16)	0.88 (0.17)
eGFR[f] (mL/min/1.73m^2)	86.2 (12.4)	85.9 (12.2)	85.7 (11.8)	86.6 (12.3)	87.0 (13.2)
Urinary albumine (mg/L)	3.6 (3.0–4.8)	3.6 (3.0–5.0)	3.6 (3.0–4.9)	3.6 (3.0–4.8)	3.6 (3.0–4.9)
UACR (mg/mmol)	0.4 (0.3–0.7)	0.5 (0.3–0.8)	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.4 (0.3–0.7)
Albuminuria (%)					
Moderately increased	2.2	3.5	2.2	2.0	1.3
Severely increased	0.8	0.0	0.9	0.9	0.7

BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RAS, renin-angiotensin system; UACR, urinary albumin to creatinine ratio.
Results were based on analyses weighted towards the BMI distribution of the general population. The number of participants with available birth weight data was 4,250.
Results are shown as mean (SD), median (IQR), or percentage in the total study population as well as stratified per birth weight category.
[a] Defined as self-reported diagnosis, or serum fasting glucose levels ≥126 mg/dL.
[b] Defined as eGFR <60.0 mL/min/1.73m^2.
[c] To convert the values for glucose to mmol/L, multiply by 0.05551.
[d] Estimated using the Friedewald formula. To convert the values for LDL-cholesterol to mmol/L, multiply by 0.02586.
[e] To convert the values for serum creatinine to µmol/L, multiply by 88.40.
[f] eGFR was estimated using the 2012 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation

Birth weight and kidney function

We found no differences in eGFR across the four birth weight categories (Table 2). After multivariable adjustment, we observed no association between birth weight and risk of CKD stage 3 or albuminuria. Restricting analyses to cases with complete data (Supplementary Table S3), or Caucasian individuals (Supplementary Table S4), did not essentially change the results.

Table 2: Difference in kidney function, urinary albumin-to-creatinine ratio, risk of CKD, and albuminuria, according to birth weight categories at age 45-65 years in 6,671 participants of the Netherlands Epidemiology of Obesity study.

Birth weight (kg)	Crude	Model 1	Model 2	Model 3
		Difference in eGFR		
<2.5	-0.91 (-2.62; 0.80)	0.21 (-1.78; 1.36)	-0.45 (-2.02; 1.12)	-0.41 (-1.98; 1.16)
2.5 to <3.0	-0.84 (-2.19; 0.52)	-0.25 (-1.50; 1.00)	-0.36 (-1.61; 0.89)	-0.37 (-1.60; 0.86)
3.0 to <4.0 (ref)	0	0	0	0
≥4.0	0.51 (-1.07; 2.10)	0.42 (-1.10; 1.93)	0.44 (-1.07; 1.95)	0.45 (-1.05; 1.95)
		Difference in UACR		
<2.5	-0.03 (-0.51; 0.46)	-0.04 (-0.53; 0.45)	-0.10 (-0.61; 0.40)	-0.10 (-0.60; 0.40)
2.5 to <3.0	0.11 (-0.28; 0.51)	0.10 (-0.30; 0.50)	0.07 (-0.32; 0.45)	0.07 (-0.31; 0.45)
3.0 to <4.0 (ref)	0	0	0	0
≥4.0	0.27 (-0.34; 0.88)	0.26 (-0.36; 0.89)	0.27 (-0.36; 0.90)	0.25 (-0.37; 0.88)
		Odds ratio for CKD [a]		
<2.5	1.18 (0.46; 3.00)	0.99 (0.39; 2.52)	0.99 (0.38; 2.54)	0.98 (0.39; 2.51)
2.5 to <3.0	1.37 (0.75; 2.50)	1.24 (0.69; 2.26)	1.24 (0.68; 2.25)	1.23 (0.69; 2.22)
3.0 to <4.0 (ref)	1	1	1	1
≥4.0	0.77 (0.36; 1.65)	0.80 (0.37; 1.74)	0.80 (0.37; 1.75)	0.78 (0.36; 1.68)
		Odds ratio for moderately or severely increased albuminuria [b]		
<2.5	1.16 (0.59; 2.30)	1.30 (0.65; 2.60)	1.21 (0.60; 2.47)	1.25 (0.61; 2.55)
2.5 to <3.0	1.14 (0.68; 1.91)	1.20 (0.72; 2.01)	1.14 (0.68; 1.91)	1.14 (0.69; 1.89)
3.0 to <4.0 (ref)	1	1	1	1
≥4.0	0.85 (0.46; 1.56)	0.77 (0.42; 1.42)	0.78 (0.41; 1.47)	0.78 (0.42; 1.46)

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; UACR, urinary albumin-to-creatinine ratio. * p<0.05.
Results were based on analyses weighted towards the BMI distribution of the general population.
Mean (SD) baseline eGFR in the reference group is 86.6 (12.3) mL/min/1.73m^2. Median (IQR) baseline UACR in the reference group is 0.45 (0.30; 0.71) mg/g.
[a] Analyses were weighted towards the BMI distribution of the general population, therefore no absolute numbers were presented. The prevalence of CKD was 2.2%.
[b] Analyses were weighted towards the BMI distribution of the general population, therefore no absolute numbers were presented. The prevalence of albuminuria was 3%.
Model 1: adjusted for age and sex.
Model 2: Model 1, additionally adjusted for race and level of education.
Model 3: Model 2, additionally adjusted for cigarette smoking and alcohol consumption.

Two-sample Mendelian randomization using individual participant data

The proportion of participants with a high birth weight (≥4000 gram) increased for each incremental quartile of the genetic risk score (Table 3). Ordinal logistic regression analyses showed that each 1-SD increase in genetic risk score was associated with a 2.9 (95% CI 1.5; 5.5, p=0.001) fold increased risk of being in a higher birth weight category. After multivariable adjustment, each 500 gram decrease in genetically determined birth weight was related to a 3.7 (95% CI: 0.5; 6.9, p=0.025) mL/min/1.73m^2 lower eGFR at middle age. The genetic risk score was not associated with age, sex, educational level, or obesity (Supplementary Table S5). Overall, the proportion of participants with diabetes was low (6%), and slightly decreased in higher quartiles of the genetic risk score. We found no relation between the genetic risk score and proteinuria: per 500 gram decrease in genetically determined birth weight the UACR decreased by 0.08 mg/mmol (p=0.8).

Table 3: According to quartiles of the genetic risk score, the proportion of participants within incremental categories of birth weight in participants of the Netherlands Epidemiology of Obesity study.

	Genetic risk score	
Birth weight (kg)	Quartile 1 (<2.17)	Quartile 4 (≥2.36)
<2.5	11.9	9.1
2.5 to <3.0	25.9	22.1
3.0 to <4.0	50.0	49.1
≥4.0	12.3	19.8

Results were based on analyses weighted towards the BMI distribution of the general Dutch population.

Two-sample Mendelian randomization using summary level data

We found no significant relation between genetically determined birth weight and creatinine based eGFR using summary level data from the CKDgen consortium (Figure 2, Table 4). The pooled effect per 1-SD genetically increased birth weight (about 500g) on log-transformed eGFR was 0.009 (-0.002; 0.019, p=0.11), which equals a 1.01% higher eGFR. Thus at middle age, each 500 gram genetically decreased birth weight was related to a 1% lower eGFR. After excluding 20 SNPs (Supplementary Table S1) with strong maternal effects, we found slightly weaker results: IVW estimator 0.004 (-0.009; 0.018, p=0.5). Leave-one-out analysis and funnel plot analysis were not suggestive for directional horizontal pleiotropy (Supplementary Figure S3 and S4). The MR-Egger intercept test indicated no directional horizontal pleiotropy (p=0.37). MR-PRESSO indicated no directional horizontal pleiotropy (p=0.20) and detected no outliers. Results of the weighted median and mode method were comparable to the IVW method (Table 4).

Table 4: Causal effect per 500 gram genetically increased birth weight on log-transformed eGFR at middle age, by different instrumental variable estimators.

Estimator	Beta	Standard error	p-value
Inverse variance weighted	0.0088	0.0055	0.11
Weighted median	0.0133	0.0072	0.06
Weighted mode	0.0157	0.0107	0.15
MR-Egger (intercept)	-0.0005	0.0006	0.37
MR-Egger (slope)	0.0253	0.0191	0.19

The Beta coefficient is the pooled causal estimate from the two-sample summary data Mendelian randomization analyses, and should be interpreted as the effect per 500 gram genetically increased birth weight on log-transformed eGFR.
MR-PRESSO analysis did not show evidence for directional horizontal pleiotropy (p=0.20), and did not detect any statistically significant (threshold p<0.05) outliers.

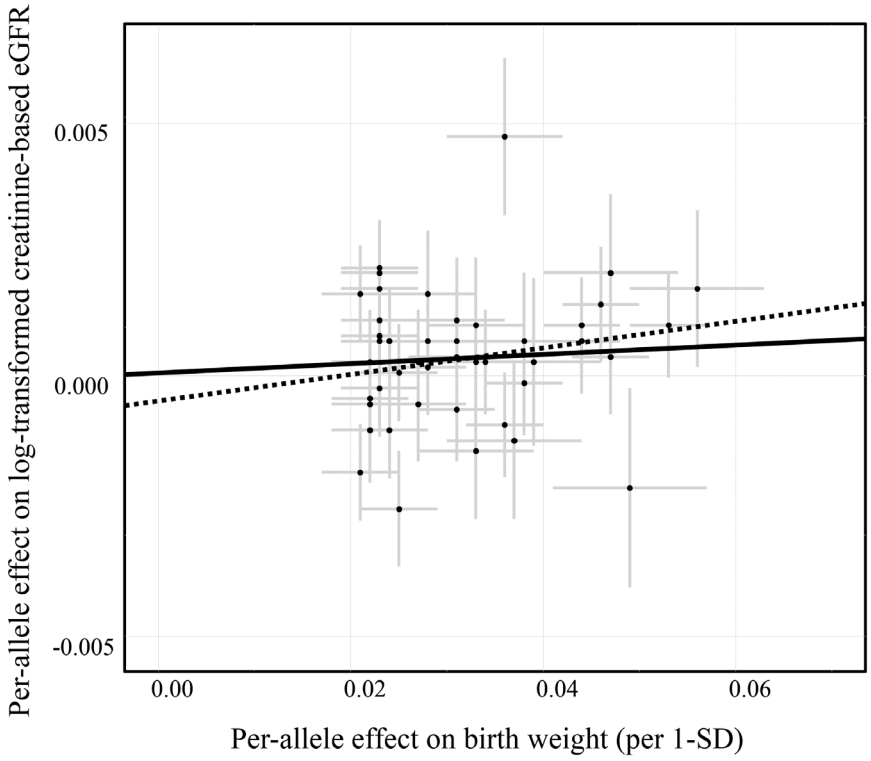

Figure 2: Per-allele effects (95%-CI) on the outcome plotted against per-allele effects (95%-CI) on the exposure. The slope of the line represents the causal association. The slope of the inverse-variance weighted line (solid line) was 0.009 (SE 0.0055, p=0.11), and for the MR-Egger (dotted line) was 0.025 (SE 0.019, p=0.19). The intercepts and slopes of the inverse-variance weighted method and MR-Egger method differ only slightly, which is confirmed by a non-significant p-value for horizontal pleiotropy (p=0.37).
MR, Mendelian randomization; SNP, single nucleotide polymorphism

DISCUSSION

In a Dutch population-based cohort of middle-aged mainly Caucasian adults, self-reported birth weight was not associated with kidney function. In contrast, two-sample Mendelian randomization analysis, showed that each 500 gram of genetically decreased birth weight was related with a 3.7 mL/min/1.73m^2 lower kidney function at middle-age in a Dutch cohort. However, we could not validate this finding in the CKDgen consortium data including 133,814 individuals, showing a small but not significant effect of genetically lower birth weight on kidney function: 1% lower eGFR per 500 gram lower birth weight.

Our results are not in line with a large meta-analysis (including 31 studies), stating that low birth weight increases the risk of CKD and ESRD.[12] However, this meta-analysis included only 2 studies representative for the general adult population. Due to high heterogeneity of included studies, suboptimal and incomplete birth weight data collection, and difficulties pooling all included studies, estimates may have been inflated.

The Nord Trøndelag Health (HUNT 2) study explored the association of birth weight with kidney function at age 20-30 years among 7,457 individuals from the general population. Its main strength was the accurate measurement of birth weight.[38] The authors of the HUNT 2 study showed that in men each additional kg of birth weight was associated with an additional eGFR increase of 1.0 (-0.1; 2.1) mL/min/1.73m^2, after adjusting for maternal factors. In women there was no association between birth weight and kidney function. The discrepancy between our results and those of the HUNT-2 study may be related to the different ages of the cohorts (20-30y *vs* 45-65y). In general, after age 40y there is an age-related annual kidney function decline of 1.0 mL/min/1.73m^2.[39, 40] In addition, risk factors such as diabetes, hypertension and smoking may accelerate kidney function decline. Taken together, the age-related kidney function decline and known risk factors of accelerated kidney function decline, may have diluted any effect of low birth weight in our older cohort of the NEO study.

Our observational cohort study has several limitations. First, birth weight of NEO-study participants was collected by means of questionnaires at the age of 45-65. Most likely, this may have led to measurement error of birth weight resulting in non-differential misclassification. In general, non-differential misclassification results in underestimation of the association between birth weight and eGFR.[41] Therefore, we performed Mendelian randomization analyses to avoid measurement error of birth weight in the NEO-study. Second, eGFR was not measured directly, but was estimated by the CKD-EPI equation, which may underestimate kidney function in participants with an eGFR higher than 90 mL/min/1.73m^2.[42] However, measured GFR is rarely available in large epidemiological studies, and even daily iothalamate measurements can vary up to 8%.[43] Third, we assessed middle-aged individuals, in whom age-related kidney function decline together with other risk factors of accelerated kidney function decline may have diluted any effect of low birth weight. Fourth, for smaller individuals, a "low" birth weight may be regarded as normal in relation to an individual's body mass and circulating volume. This is not taken into account by currently used absolute cut-offs for low birth weight. Finally, we had no information about confounding factors such as gestational age, and lifestyle during pregnancy such as cigarette smoking, alcohol use, and malnutrition.

These factors are important causes of low birth weight, and not taking them into account could lead to overestimation of a potential negative effect of low birth weight. However, in our study we found no relevant association between low birth weight and kidney function.

Limitations of our Mendelian randomization analyses are mainly related to the used instrument. First, the GWAS investigating SNPs associated with birth weight excluded individuals with a birth weight <2.5 kg and >4.5 kg from part of the used data sources. This may have resulted in exclusion of SNPs associated with low or high birth weight. Second, some of the SNPs could have an effect via a maternal pathway, rather than direct fetal effects on birth weight. However, Horikoshi *et al.* showed that the fetal genetic variation had a greater impact on birth weight than maternal variation at 55/59 genetic loci.[13] Excluding SNPs with strong maternal effects did not change our results. Third, the Mendelian randomization assumption of no directional horizontal pleiotropy requires that an instrument affects the outcome only via the exposure of interest (birth weight), and not via other mechanisms. An instrument consisting of 59 different SNPs may therefore be particularly prone to directional horizontal pleiotropy. However, MR-Egger and MR-PRESSO analyses showed no evidence for directional horizontal pleiotropy. Fourth, our genetic instrument explained only 2% of the birth weight variance, which may result in limited power. Importantly, confidence intervals were informative both in the NEO-study and in the CKDgen data, which implies sufficient power in both cases. Fifth, using many genetic instruments increases the risk of weak instrument bias. If an instrument is weak, any association between the instrument and the outcome may be explained by unbalanced confounders, rather than by the instrument itself.[30] In the present study, the instruments were chosen based on a large-scale independent genome-wide association study on birth weight, which is reflected by the high F-statistics (F >10). Of note, patient overlap between populations may hamper the interpretation of bias in case of weak instruments. In the present study, there was 2.8% overlap of participants between Horikoshi *et al.* and the NEO study, and 4.6% between Horikoshi *et al.* and the CKDgen consortium. Given the relatively small proportion of overlap, and the sufficiently strong instruments that we used, any influence of weak instrument bias is most likely negligible.

The most important strength of our study is that we used three complementary approaches.- We performed an observational study with a large sample, representative for the general population. For the two-sample Mendelian randomization analyses we used an instrument based on a previously validated genetic score for birth weight, and we validated our findings in the NEO-study using summary level data of the CKDgen consortium.

Self-reported birth weight was not associated with kidney function. In contrast, each 500 gram of genetically decreased birth weight was related with a 3.7 mL/min/1.73m^2 lower kidney function at middle-age in a Dutch cohort. However, we could not validate this finding in another cohort of the CKDgen consortium.

ARTICLE INFORMATION

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AUTHORS' CONTRIBUTIONS

Research idea and study design: EH, KE, AV, RM, FR, DM; data acquisition: KE, RM, FR, DM, TR; data analysis/interpretation: KE, EH, DM, RM, FR, RS, JF; statistical analyses: KE, EH, DM; RS supervision or mentorship: EH, DM, JF. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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SUPPLEMENTARY DATA

Table S1: Genetic variants (n=59) associated with birth weight adapted from the genome-wide association study by Horikoshi *et al.*[1] We included these variants in a genetic risk score that we used as instrument for birth weight.

SNP		Gene	Chr	ffect/other allele	Beta*	SE	EAF	R^2 (%) **	F-stat ***
rs2473248		WNT4-ZBTB40	1	C/T	0.033	0.006	0.87	0.024	33.3
rs3753639	b	ZBTB7B	1	C/T	0.031	0.004	0.23	0.034	47.2
rs72480273		FCGR2B	1	C/A	0.031	0.005	0.36	0.028	38.3
rs61830764	c	DTL	1	A/G	0.022	0.004	0.17	0.023	31.5
rs7575873		ATAD2B	2	A/G	0.038	0.006	0.88	0.031	43.4
rs1374204	d	EPAS1	2	T/C	0.047	0.004	0.70	0.093	124.9
rs2242116	d	PTH1R	3	A/G	0.022	0.004	0.39	0.022	31.6
rs11719201	b	ADCY5	3	T/C	0.046	0.004	0.23	0.076	109.2
rs10935733		CPA3	3	T/C	0.022	0.004	0.42	0.024	33.0
rs13322435	d	CCNL1-LEKR1	3	A/G	0.053	0.004	0.59	0.136	190.4
rs925098	d	LCORL	4	G/A	0.034	0.004	0.28	0.046	63.6
rs6537307	d	HHIP	4	G/A	0.025	0.004	0.48	0.032	45.0
rs854037		5q11.2	5	A/G	0.027	0.005	0.80	0.022	30.8
rs7729301	d	EBF1	5	A/G	0.024	0.004	0.72	0.023	32.4
rs35261542		CDKAL1	6	C/A	0.044	0.004	0.73	0.078	111.7
rs9379832	c	HIST1H2BE	6	A/G	0.023	0.004	0.71	0.022	30.4
rs7742369	b	HMGA1	6	G/A	0.028	0.005	0.19	0.024	32.4
rs1415701	d	L3MBTL3	6	G/A	0.025	0.004	0.73	0.025	35.4

Table S1: Contiued

SNP			Gene	Chr	ffect/other allele	Beta*	SE	EAF	R^2 (%) **	F-stat ***
rs1101081			ESR1	6	C/T	0.038	0.004	0.73	0.057	79.5
rs798489		d	GNA12	7	C/T	0.023	0.004	0.74	0.021	30.8
rs11765649			IGF2BP3	7	T/C	0.027	0.004	0.76	0.027	37.3
rs6959887	b		TBX20	7	A/G	0.023	0.004	0.61	0.025	35.5
rs138715366	c	d	YKT6-GCK	7	C/T	0.241	0.023	0.99	0.103	136.0
rs62466330			MLXIPL	7	C/T	0.049	0.008	0.07	0.030	42.0
rs13266210			ANK1-NKX6-3	8	A/G	0.031	0.005	0.79	0.031	43.9
rs6989280		d	TRIB1	8	G/A	0.022	0.004	0.70	0.019	26.9
rs12543725		d	SLC45A4	8	G/A	0.023	0.004	0.60	0.026	36.0
rs28510415		d	PTCH1	9	G/A	0.056	0.007	0.09	0.052	70.6
rs2150052	c	d	LPAR1	9	T/A	0.021	0.004	0.50	0.022	31.0
rs7847628	b		PHF19	9	G/A	0.023	0.004	0.67	0.024	32.9
rs700059			STRBP	9	G/A	0.033	0.005	0.16	0.027	37.5
rs61862780			HHEX-IDE	10	T/C	0.028	0.004	0.52	0.039	56.7
rs74233809			NT5C2	10	C/T	0.037	0.007	0.08	0.020	28.7
rs7076938			ADRB1	10	T/C	0.036	0.004	0.73	0.052	74.7
rs2421016		d	PLEKHA1	10	T/C	0.021	0.004	0.48	0.021	30.8
rs72851023	c		INS-IGF2	11	T/C	0.048	0.008	0.07	0.031	41.6
rs10830963	a	d	MTNR1B	11	G/C	0.023	0.004	0.27	0.022	31.2
rs11055034			APOLD1	12	C/A	0.022	0.004	0.73	0.019	27.4
rs139975827	c		ABCC9	12	G/A	0.025	0.004	0.63	0.029	35.7

Table S1: Contiued

SNP			Gene	Chr	ffect/other allele	Beta*	SE	EAF	R^2 (%) **	F-stat ***
rs12823128			ITPR2	12	T/C	0.021	0.004	0.56	0.022	30.8
rs1351394		d	HMGA2	12	T/C	0.044	0.004	0.48	0.095	136.6
rs7964361			IGF1	12	A/G	0.039	0.007	0.08	0.024	33.2
rs2324499	b		LINC00332	13	G/C	0.022	0.004	0.67	0.020	28.6
rs2854355			RB1	13	G/A	0.023	0.004	0.26	0.022	29.7
rs1819436			RNF219-AS1	13	C/T	0.033	0.006	0.87	0.024	34.0
rs12906125	b	d	FES	15	G/A	0.023	0.004	0.69	0.023	32.0
rs7402982	b	d	IGF1R	15	A/G	0.023	0.004	0.42	0.026	36.8
rs1011939	c		GPR139	16	G/A	0.022	0.004	0.31	0.020	28.4
rs113086489		d	CLDN7	17	T/C	0.031	0.004	0.55	0.046	64.8
rs144843919	c		SUZ12P1-CRLF3	17	G/A	0.066	0.012	0.96	0.029	35.7
rs12942207			SP6-SP2	17	C/T	0.022	0.004	0.30	0.021	29.4
rs61154119		d	ACTL9	19	T/G	0.028	0.005	0.84	0.021	27.5
rs10402712	c		PEPD	19	A/G	0.022	0.004	0.27	0.018	24.8
rs6040076	c		JAG1	20	C/G	0.023	0.004	0.51	0.027	37.2
rs28530618	c		C20orf203	20	A/G	0.026	0.004	0.50	0.034	47.1
rs6016377			MAFB	20	T/C	0.024	0.004	0.45	0.028	39.1
rs2229742	a		NRIP1	21	G/C	0.036	0.006	0.87	0.029	42.1
rs134594			KREMEN1	22	C/T	0.023	0.004	0.35	0.023	32.2
rs62240962			SREBF2	22	C/T	0.047	0.007	0.92	0.012	16.3

Chr, chromosome; SNP, single-nucleotide polymorphism; SE, standard error; EAF, effect allele frequency
* per allele effect on birth weight per SD: 1 SD is about 500 gram birth weight.
** explained variance in birth weight (%): $R^2 = 2 \cdot \beta^2 \cdot EAF \cdot (1-EAF)$ [2]

*** calculated as follows: $F = \frac{N-K-1}{K} \bullet \frac{R^2}{1-R^2}$ (where N = sample size, derived from the supplemental data in Horikoshi *et al.*, and K = number of SNPs, e.g. K = 1 if single SNPs are tested) [2]
[a] SNP not available in NEO study (n=2)
[b] SNP not available in CKDgen data, proxy used (n=8), proxies are specified in Supplementary Table 2
[c] SNP not available in CKDgen data and no proxy found (n=11)
[d] SNP excluded in sensitivity analysis, based on statistically significant maternal effects, as reported in the supplemental data in Horikoshi *et al.* (n=20). The threshold for statistical significance was a Bonferroni corrected p-value of 0.0011, based on 45 SNPs.

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Table S2: List of used proxies for genetic variants not available in the CKDgen data. Only variants with an R^2>0.80 were selected.

SNP	Proxy	Gene	Chr	Effect/other allele	EAF	R^2
rs3753639	rs905938	ZBTB7B	1	C/T	0.27	0.876
rs11719201	rs11708067	ADCY5	3	G/A	0.20	0.950
rs7742369	rs1776877	HMGA1	6	G/A	0.16	1.000
rs6959887	rs988270*	TBX20	7	C/T	0.68	0.962
rs7847628	rs3933326*	PHF19	9	G/A	0.63	0.895
rs2324499	rs7998537*	LINC00332	13	G/A	0.68	0.962
rs12906125	rs6227	FES	15	C/T	0.63	0.965
rs7402982	rs2017500	IGF1R	15	G/A	0.45	0.935

Chr, chromosome; EAF, effect allele frequency; SNP, single-nucleotide polymorphism
* Removed from analysis because in LD (R^2>0.001) with other SNP.

Table S3: According to four birth weight categories, difference in kidney function, urinary albumin-to-creatinine ratio, risk of CKD, and albuminuria, at age 45-65 years in 6,671 participants of the Netherlands Epidemiology of Obesity study, including only complete cases.

Birth weight (kg)	Crude	Model 1	Model 2	Model 3
		Difference in eGFR		
<2.5	-0.25 (-2.11; 1.61)	-0.04 (-1.81; 1.73)	-0.18 (-1.97; 1.61)	-0.10 (-1.90; 1.70)
2.5 to <3.0	-0.56 (-1.82; 0.70)	-0.38 (-1.61; 0.85)	-0.44 (-1.68; 0.80)	-0.42 (-1.66; 0.81)
3.0 to <4.0 (ref)	0	0	0	0
≥4.0	0.70 (-0.78; 2.18)	0.10 (-1.30; 1.51)	0.19 (-1.21; 1.59)	0.20 (-1.20; 1.59)
		Difference in UACR		
<2.5	-0.16 (-0.34; 0.02)	-0.14 (-0.30; 0.01)	-0.20 (-0.37; 0.03)	-0.20 (-0.37; -0.02)*
2.5 to <3.0	0.09 (-0.29; 0.46)	0.10 (-0.27; 0.46)	0.08 (-0.31; 0.46)	0.09 (-0.30; 0.47)
3.0 to <4.0 (ref)	0	0	0	0
≥4.0	0.34 (-0.42; 1.10)	0.35 (-0.41; 1.12)	0.38 (-0.39; 1.15)	0.36 (-0.41; 1.12)
		Odds ratio for CKD [a]		
<2.5	0.86 (0.29; 2.53)	0.81 (0.26; 2.46)	0.79 (0.26; 2.39)	0.79 (0.26; 2.37)
2.5 to <3.0	0.98 (0.50; 1.94)	1.02 (0.52; 2.02)	1.01 (0.51; 2.01)	1.02 (0.51; 2.02)
3.0 to <4.0 (ref)	1	1	1	1
≥4.0	0.60 (0.25; 1.43)	0.73 (0.31; 1.74)	0.73 (0.31; 1.74)	0.72 (0.30; 1.71)
		Odds ratio for moderately or severely increased albuminuria [b]		
<2.5	1.25 (0.67; 2.32)	1.48 (0.79; 2.78)	1.40 (0.74; 2.64)	1.47 (0.77; 2.79)
2.5 to <3.0	1.08 (0.63; 1.83)	1.19 (0.70; 2.02)	1.17 (0.70; 1.98)	1.17 (0.70; 1.96)
3.0 to <4.0 (ref)	1	1	1	1
≥4.0	0.68 (0.37; 1.26)	0.64 (0.34; 1.21)	0.65 (0.34; 1.24)	0.66 (0.35; 1.26)

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; UACR, urinary albumin to creatinine ratio. * p<0.05. Results were based on analyses weighted towards the BMI distribution of the general population. Mean (95%-CI) baseline eGFR in the reference group is 86.5 (85.8; 87.3) mL/min/1.73m^2. Mean (95%-CI) baseline UACR in the reference group is 0.82 (0.66; 0.99) mg/g.

[a] Analyses were weighted towards the BMI distribution of the general population, therefore no absolute numbers were presented. The prevalence of CKD was 2.2%.

[b] Analyses were weighted towards the BMI distribution of the general population, therefore no absolute numbers were presented. The prevalence of albuminuria was 3%.

Model 1: adjusted for age and sex.

Model 2: Model 1, additionally adjusted for race and level of education.

Model 3: Model 2, additionally adjusted for cigarette smoking and alcohol consumption.

Table S4: According to four birth weight categories, difference in kidney function, urinary albumin-to-creatinine ratio, risk of CKD, and albuminuria, at age 45-65 years restricted to Caucasian participants (95% of the cohort) of the Netherlands Epidemiology of Obesity study.

Birth weight (kg)	Crude	Model 1	Model 2	Model 3
		Difference in eGFR		
<2.5	-0.86 (-2.58; 0.86)	-0.06 (-1.67; 1.55)	-0.14 (-1.75; 1.47)	-0.11 (-1.71; 1.50)
2.5 to <3.0	-0.68 (-2.08; 0.72)	-0.08 (-1.37; 1.21)	-0.15 (-1.45; 1.14)	-0.14 (-1.42; 1.13)
3.0 to <4.0 (ref)	0	0	0	0
≥4.0	0.67 (-0.90; 2.24)	0.56 (-0.95; 2.06)	0.57 (-0.92; 2.06)	0.57 (-0.90; 2.05)
		Difference in UACR		
<2.5	-0.03 (-0.57; 0.52)	-0.05 (-0.60; 0.51)	-0.07 (-0.62; 0.48)	-0.07 (-0.62; 0.48)
2.5 to <3.0	-0.01 (-0.34; 0.22)	-0.02 (-0.36; 0.31)	-0.05 (-0.38; 0.28)	-0.04 (-0.37; 0.29)
3.0 to <4.0 (ref)	0	0	0	0
≥4.0	0.28 (-0.35; 0.91)	0.26 (-0.38; 0.91)	0.27 (-0.38; 0.91)	0.25 (-0.39; 0.89)
		Odds ratio for CKD [a]		
<2.5	1.23 (0.47; 3.21)	1.01 (0.38; 2.65)	0.99 (0.37; 2.61)	0.98 (0.37; 2.58)
2.5 to <3.0	1.37 (0.74; 2.53)	1.24 (0.68; 2.26)	1.23 (0.67; 2.25)	1.22 (0.67; 2.22)
3.0 to <4.0 (ref)	1	1	1	1
≥4.0	0.76 (0.34; 1.66)	0.79 (0.36; 1.74)	0.79 (0.36; 1.75)	0.77 (0.35; 1.69)
		Odds ratio for moderately or severely increased albuminuria [b]		
<2.5	1.26 (0.65; 2.44)	1.39 (0.71; 2.73)	1.35 (0.69; 2.64)	1.39 (0.70; 2.73)
2.5 to <3.0	1.11 (0.64; 1.94)	1.17 (0.67; 2.04)	1.13 (0.65; 1.95)	1.13 (0.66; 1.94)
3.0 to <4.0 (ref)	1	1	1	1
≥4.0	0.83 (0.45; 1.54)	0.75 (0.40; 1.40)	0.76 (0.40; 1.42)	0.76 (0.40; 1.43)

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; UACR, urinary albumin to creatinine ratio. * p<0.05. Results were based on analyses weighted towards the BMI distribution of the general population. Mean (95%-CI) baseline eGFR in the reference group is 86.5 (85.8; 87.3) mL/min/1.73m^2. Mean (95%-CI) baseline UACR in the reference group is 0.82 (0.66; 0.99) mg/g.

[a] Analyses were weighted towards the BMI distribution of the general population, therefore no absolute numbers were presented. The prevalence of CKD was 2.2%.

[b] Analyses were weighted towards the BMI distribution of the general population, therefore no absolute numbers were presented. The prevalence of albuminuria was 3%.

Model 1: adjusted for age and sex.

Model 2: Model 1, additionally adjusted for race and level of education.

Model 3: Model 2, additionally adjusted for cigarette smoking and alcohol consumption.

Table S5: Proportion of risk factors for CKD according to quartiles of the genetic risk score for birth weight, in participants of the Netherlands Epidemiology of Obesity study.

	Proportion of participants with risk factor for CKD, per quartile of genetic risk score for birth weight			
	Quartile 1 (<2.17)	Quartile 2 (2.17 to 2.26)	Quartile 3 (2.27 to 2.35)	Quartile 4 (≥2.36)
Women	53.8	55.3	57.5	57.2
≥55 years	56.2	58.6	58.6	57.2
Lower education	52.2	52.4	54.2	51.6
BMI ≥30.0 kg/m^2	13.6	16.6	15.7	16.3
Diabetes	6.3	5.9	5.0	4.3

BMI, body mass index; CKD, chronic kidney disease.
Results were based on analyses weighted towards the BMI distribution of the general Dutch population.

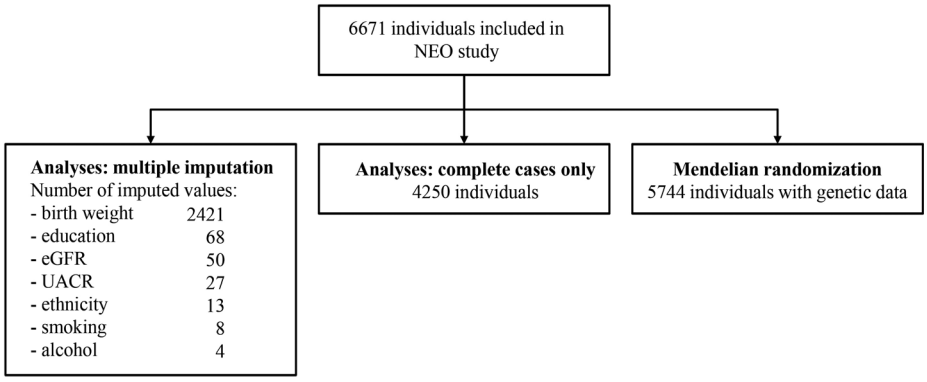

Figure S1: Flow chart of 6,671 participants of the Netherlands Epidemiology of Obesity study.

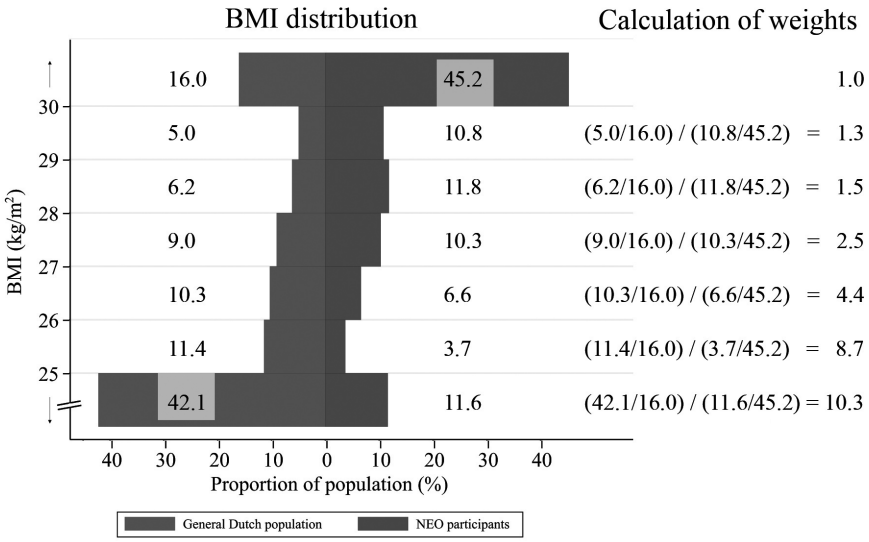

Figure S2: BMI distribution of the NEO participants (blue) compared to the general Dutch population (red), and derivation of the weights for weighted analyses. Owing to the oversampling of overweight individuals, the BMI distribution of NEO participants substantially deviates from the general population. For generalizability purposes, analyses in NEO participants were weighted towards the distribution of the general population. For example, the weight for analysis in NEO participants with a BMI< 25 kg/m² was calculated as follows: in NEO participants the ratio of those with a BMI <25 kg/m² compared to those with a BMI >30 kg/m² was 11.6/45.2=0.257. In the general population this ratio was 42.1/16.0=2.63. The weight for participants of the NEO study with BMI <25 kg/m² was then 2.63/0.257=10.3. In this manner, the BMI distribution of NEO participants (blue) becomes similar to the general population (red).

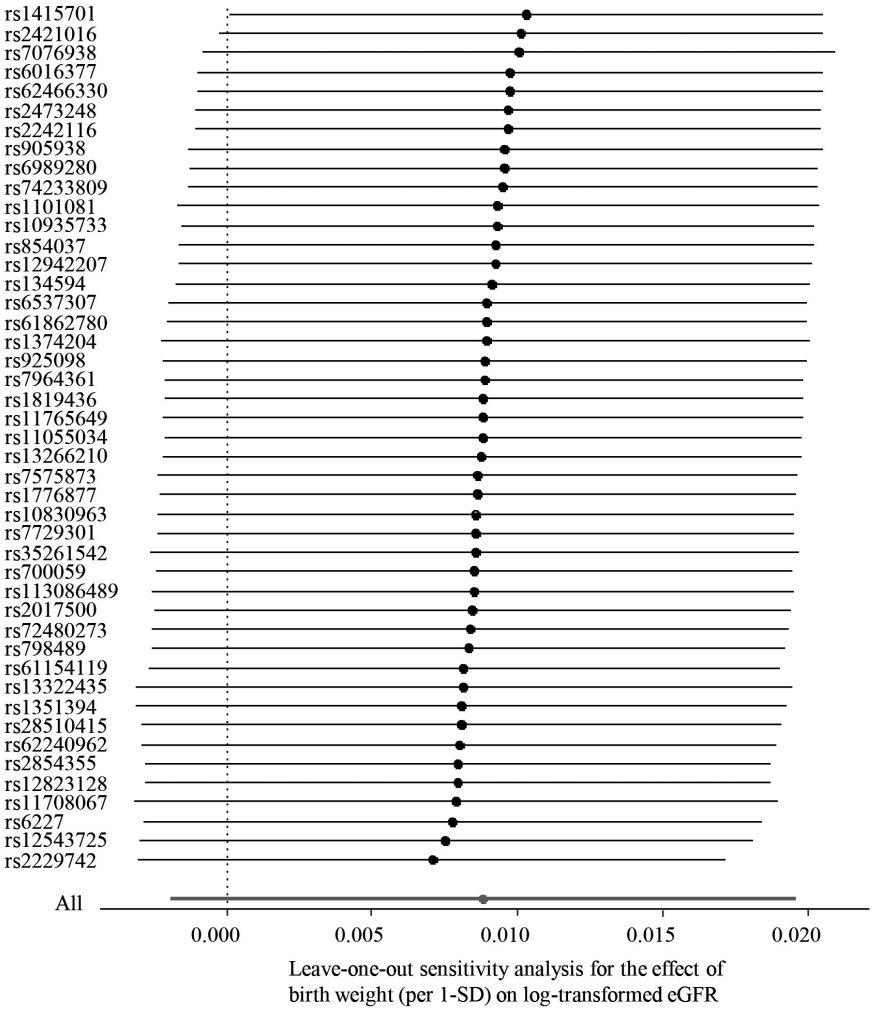

Figure S3: Leave-one-out sensitivity analysis to identify potential influential outliers. For each SNP the summary effect estimate is plotted after excluding a single SNP. In case of influential outliers, leaving the single SNP out, may result in a large deviation of the effect estimate, compared to the overall effect estimate of all SNPs. In the present study, all effect estimates excluding one single SNP are roughly comparable to the overall effect including all SNPs. Therefore, this analysis is not suggestive for pleiotropy owing to outliers.

eGFR, estimated glomerular filtration rate; SD, standard deviation; SNP, single-nucleotide polymorphism.

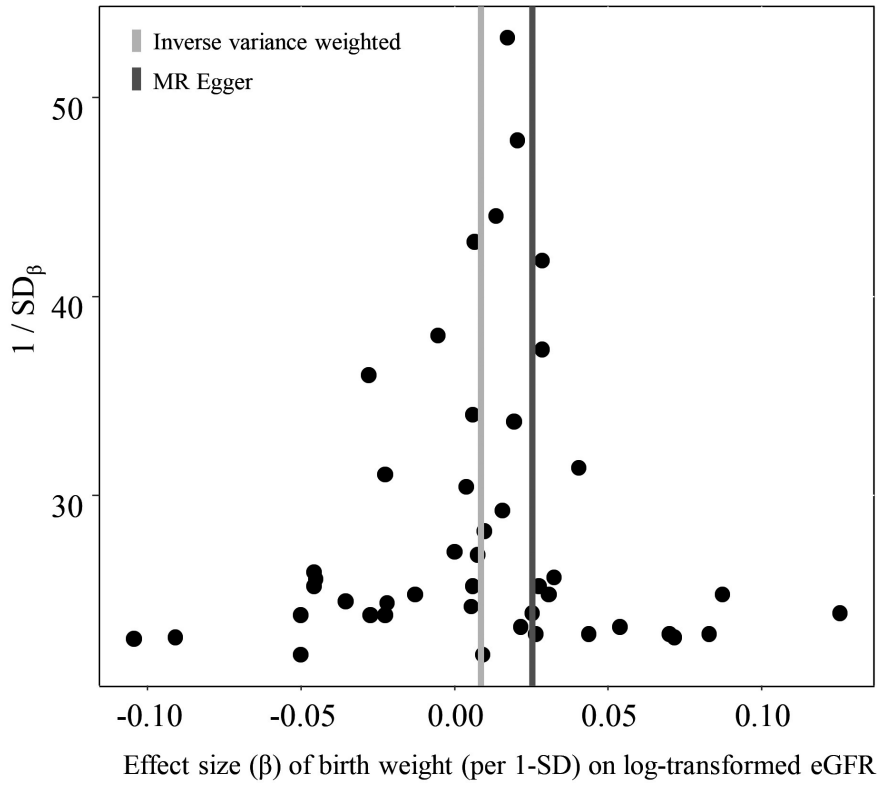

Figure S4: Funnel plot analysis to detect directional pleiotropy. For each SNP, the causal estimate (β) is plotted against the precision of the causal estimate. Asymmetry may arise when certain SNPs have very strong effects on the outcome, which may indicate directional horizontal pleiotropy. In the present study, the funnel plot is symmetrical, which is reflected also by the non-significant MR-Egger intercept test (p=0.37).
eGFR, estimated glomerular filtration rate; SD, standard deviation; SNP, single-nucleotide polymorphism.

Chapter 9 –

Discussion

In this thesis we aimed to investigate the role of a variety of risk factors for chronic kidney disease (CKD) progression, mainly focused on patients at high cardiovascular risk. These risk factors encompass both traditional cardiovascular risk factors, as well as lifestyle factors such as obesity and diet, acute kidney injury, and the role of low birth weight. Research on (modifiable) risk factors in cardiovascular compromised patients is relatively scarce. The trends of population ageing and unhealthier lifestyle, lead to a growing population with CKD, which is the rationale of this thesis. Additionally, we assessed the beneficial renal effects of use of cholesterol-lowering medication (statins) for secondary prevention. Globally, statins are among the most prescribed drugs, also in CKD patients. We therefore not only investigated the effect of statins as a whole, but additionally aimed to assess whether certain types of statins may be preferable from a renal perspective. This chapter briefly discusses the main findings of this thesis, and incorporates these into clinical implications and recommendations.

MAIN FINDINGS IN CONTEXT

In **Chapter 2, 3, and 4**, we investigated the role of different risk factors on CKD progression in post-myocardial infarction (MI) patients of the Alpha Omega Cohort. In **Chapter 2** we showed that diabetes and hypertension are the strongest drivers for the accelerated kidney function decline in post-MI patients, which in is line with previous publications.[1–3] To a lesser extent, obesity and smoking were also associated with faster kidney decline. Most importantly, we found that patients with a higher compared to lower number of cardiovascular risk factors have a faster progression of kidney function decline. Post-MI patients with optimally treated cardiovascular risk factors had an annual eGFR decline of 0.90 mL/min/1.73m^2, which is comparable to the general population. In contrast, patients with at least three cardiovascular risk factors had a three-fold faster rate of kidney function decline. In **Chapter 3** we investigated the role of obesity more in detail. Both body mass index and waist circumference were associated with faster eGFR decline. Our results underline the importance of a healthy weight, as recommended in current guidelines, and argue against the so called "obesity paradox".[4] The obesity paradox propagates for a variety of chronic diseases that overweight and obesity compared to normal weight lead to improved survival.[5, 6] However, this phenomenon is based on selection bias, and such results should be interpreted with caution.[7] In **Chapter 4** we showed a strong linear relation between protein intake and faster eGFR decline. Our findings are in agreement with current KDIGO guidelines, which recommend

to limit daily total protein intake to <1.30 g/kg body weight, and restrict intake to 0.60-0.80 g/kg per day in patients with diabetes or CKD stage 4B or higher.[4] Our results were in line with results from the Singapore Chinese Health Study.[8] However, in several Dutch and US community based cohorts no association was found between dietary protein intake and CKD risk.[9-11] Importantly, participants in the latter studies were relatively healthy and 20 years younger than the post-myocardial infarction patients described in this thesis. We found comparable associations for dietary protein from animal and plant sources, thereby not supporting the hypothesis that protein derived from plant sources is healthier compared to animal sources.

In **Chapter 5** we showed that for type 1 diabetes patients with end-stage renal disease (ESRD) a simultaneous pancreas-kidney transplantation led to 15% and 33% reduced 10-year mortality compared to those who received only a kidney transplantation from a living or deceased donor. Previous studies showed that a simultaneous pancreas-kidney transplantation is associated with improved survival compared to a kidney transplant alone from a deceased donor.[12, 13] However, only a few studies compared survival after a simultaneous pancreas-kidney transplantation with a kidney transplant alone from a living donor. Although a recent study showed that the 10-year survival benefit for a pancreas-kidney transplantation compared to a kidney transplant alone from a living donor is clinically irrelevant,[14] the majority of studies showed that short-term survival was similar and long-term survival was better in patients receiving both a pancreas and kidney, compared to a kidney from a living donor.[15] Moreover, using regional differences in preferred treatment, we showed that a treatment strategy that preferably transplants both a pancreas and kidney resulted in a 44% reduced 10-year mortality compared to a treatment strategy that favoured transplantation of a kidney alone.

In **Chapter 6** the renal effects of statins were examined, in a pair-wise and network meta-analysis of randomized controlled trials. In pair-wise meta-analysis, pooling all statins, statins compared to control treatment led to a 0.57 mL/min/1.73m^2 reduction in annual eGFR decline. These figures are comparable to another meta-analysis from 2016.[16] Likewise, statins compared to control led to a small reduction in proteinuria after one year, although in this case there was significant evidence for publication bias. In a subsequent network meta-analysis, generally all statins performed better than control, though confidence intervals were very wide and substantially overlapped. Due to a lack of power, it is therefore impossible to draw firm conclusions of superiority of certain statins regarding CKD progression.

In **Chapter 7** we addressed the potential of two novel biomarkers, TIMP-2 and IGFBP7, for the early diagnosis of acute kidney injury (AKI) in patients

undergoing elective cardiac surgery. Cardiac surgery may cause an episode of AKI, which increases the risk of CKD and mortality. We found that both biomarkers were at most of minor added value in the early prediction of AKI after elective cardiac surgery.

In **Chapter 8** we investigated the Brenner hypothesis, in relation to kidney function.[17] According to this hypothesis individuals with low compared to normal birth weight develop less glomeruli, making them more susceptible to develop CKD later in life. In the Netherlands Epidemiology of Obesity (NEO) study, we found no evidence of an association between self-reported birth weight and kidney function at middle age. Two-sample Mendelian randomization analyses, using a genetic score for birth weight, showed a 3.8 mL/min/1.73m^2 lower kidney function per 500 gram genetically lower birth weight at middle age in the NEO study.[18] In two-sample Mendelian randomization analyses in 133,814 individuals from the CKDgen consortium, we found that each 500 gram genetically decreased birth weight was non-significantly associated with a 1% lower eGFR.[19] We thus concluded that the effect of low birth weight on kidney function at middle age is small. Our results are different from a meta-analysis of 31 studies, showing that low birth weight was associated with a 70% higher risk to develop CKD.[20] Importantly, the included studies consisted of highly selected populations, not representative for the general population. The HUNT-2 study explored the association between birth weight and kidney function in 7457 individuals aged 20-30y, and measured birth weight accurately, using birth weight registry data.[21] They found a relatively small effect of low birth weight only in men, which disappeared after adjustment for maternal factors. Importantly, all previous studies were observation cohort studies, and were therefore sensitive to confounding. Our study was the first to use Mendelian randomization analyses to address the association between birth weight and kidney function.

Limitations and strengths of this research

In each chapter the main study limitations and strengths are reported. In the current section, we therefore report the general limitations of the research described in this thesis, and a brief overview of strengths per chapter.

Limitations

First, observational research is sensitive to confounding, resulting from differences in patient characteristics with regard to the exposure of interest. Apart from the analyses in **Chapter 6**, all chapters report the results of observational cohort studies. Due to the non-randomized nature of observational studies, patients in one stratum of the exposure are usually not similar to, or

exchangeable with, patients in another stratum. Lack of exchangeability may lead to incorrect results, because any association between the exposure and outcome of interest may in fact be wholly or partly explained by other factors for which groups based on the exposure differ.[22] The effect of such confounding may be reduced by adjusting the analyses for factors that differ across strata of the exposure, and are also associated with the outcome. However, one can never be sure that all confounding is corrected, e.g. because confounders may have been unmeasured or unknown, or they may have been measured imprecisely. In the present thesis, although all reported research was adjusted for the most important confounding factors, we can therefore not exclude the possibility of any residual confounding. Notably, often observational research is more feasible than performing a randomized controlled trial, in terms of time, costs, and ethics. For example, investigating the effect of obesity or low birth weight is no possible in a randomized controlled trial. It is both practically infeasible and ethically objectionable to allocate the exposure "obesity" or "low birth weight" to a patient group. Therefore, the limitations of observational research should be acknowledged balanced against the benefits.

Second, for several chapters we measured data by questionnaires. In **Chapter 4** validated food frequency questionnaires were used to measure dietary intake. In **Chapter 8** birth weight was collected using questionnaires. Furthermore, data on comorbidity, medical history, and medication use is often collected by questionnaires. Though in general questionnaires yield valid results, depending on the questions they are sensitive to measurement error, recall bias, and missing data. In **Chapter 8** we showed that a large proportion of birth weight data was missing. Using food frequency questionnaires to measure food intake may result in under- or overestimation of food intake.[23] However, when conducting a study including many patients, questionnaires are often preferred and may even be the only possibility, both in terms of time, cost-effectiveness and logistics.

Strengths

In **Chapter 2, 3, and 4** we used data from the Alpha Omega Cohort, which is the largest post-myocardial infarction patient cohort to date. Additionally, due to very strict data collection, the number of missing data was negligible. The Alpha Omega Cohort therefore provided an ideal opportunity to investigate potential modifiable risk factors for kidney function decline in patients at high cardiovascular risk. In **Chapter 5** we used registry data of all type 1 diabetes patients requiring renal replacement therapy in the Netherlands over a 30-year follow-up period. The nationwide nature of the data prevented any selective in- or exclusion of patients. Moreover, we used regional differences in treatment

strategies within The Netherlands. Using an intention-to-treat like analysis, we aimed to maximally reduce the influence of confounding. In **Chapter 6** we used state-of-the-art methodology to conduct a network meta-analysis on the effect of statins on renal outcomes, to provide evidence on which statins should be preferred from a kidney perspective. Network meta-analyses incorporate both direct and indirect evidence of all connections in a treatment network, to provide a hierarchical overview of all treatments. In **Chapter 7** we used data of a large cohort of elective cardiac surgery patients, to investigate the potential value of two novel urinary biomarkers in the prediction of acute kidney injury. In addition to estimating discrimination of both biomarkers univariably, as is done in most current publications, we assessed the added value to simple multivariable models. Finally, in **Chapter 8**, we used three different methods and three different data sources, to investigate the effect of low birth weight on kidney function at middle age. For two analyses we used as instrumental variable for birth weight an instrument based on 59 genetic variants that were associated with birth weight in a previously published genome-wide association study.

CONCLUSIONS, IMPLICATIONS AND RECOMMENDATIONS

Cardiovascular and lifestyle risk factors in cardiovascular patients

With this thesis, we provide nuance in the general idea that post-myocardial infarction patients have compared to the general population have a two-fold faster kidney function decline. We showed that, depending on the number of risk factors, kidney function decline may be comparable to the average decline in the general population. We furthermore found that diabetes and hypertension are the most important drivers of CKD progression. Therefore, we recommend that optimization of these, and other, risk factors is important to prevent CKD progression. We showed that obesity is a risk factor rather than a protective factor in post-MI patients, which underlines current KDIGO guidelines recommending an ideal body mass index lower than 25 kg/m^2.

Furthermore, dietary protein restriction is a potentially effective preventive intervention. Importantly, since nutrients are part of a dietary pattern, simply reducing intake of one component such as protein is unrealistic. Randomized controlled intervention studies evaluating a dietary pattern as a whole, for a timespan of several years, would provide the most solid evidence on the effectiveness of dietary interventions. Notably, such studies are complex. First, defining the interventions is difficult, since these may vary from person to person. More importantly, since blinding of participants is difficult

to maintain, there may be contamination in the control groups. Patients are willing to participate in the study to become healthier, increasing the chance that patients randomized to the control intervention will change their behaviour nonetheless.[24] Finally, since dietary pattern is difficult to change, compliance may pose a problem, especially over longer periods of time. Despite these challenges in the design of nutritional intervention studies, nutrition is warranted to play an increasingly important role in the prevention of chronic (cardiovascular) diseases.

Finally, we showed that prescribing a statin for cardiovascular prevention, led to slower annual eGFR decline and a reduction of proteinuria. However, we cannot provide a strong recommendation as to which statin should preferably be prescribed to attenuate CKD progression. In line with our results, current KDIGO guidelines recommend a statin in all non-dialysis dependent CKD patients 50y and older with an eGFR lower than 60 mL/min/1.73m^2, or at least 30 mg/g albuminuria, independent of serum cholesterol levels.[25] Finally, KDIGO guidelines do not specify which statin should be used, which is underlined by our study.[25]

Transplantation in type 1 diabetes patients with ESRD

We showed that type 1 diabetes mellitus patients with renal failure who received a simultaneous pancreas-kidney transplantation had the best survival, compared to patients who received a kidney transplantation alone. The difference was most pronounced compared to a kidney from a deceased donor, but 10-year survival in pancreas-kidney transplanted patients was also 15% better compared to patients receiving a living donor kidney. In general, a treatment strategy with a preference for simultaneous pancreas-kidney transplantation, rather than a kidney transplantation alone, resulted in a 44% and 31% lower 10- and 20-year mortality risk. For type 1 diabetes patients with ESRD, a simultaneous pancreas-kidney transplantation should therefore be the first choice.

Prediction of AKI

The relatively novel urinary biomarkers TIMP-2 and IGFBP7 appeared of minor value in the prediction of AKI in a relatively healthy ICU population of elective cardiac surgery patients. Our results do not argue against the use of these biomarkers in general ICU populations. However, in elective cardiac surgery patients, both markers poorly predicted AKI stage 2 or 3, and at best moderately predicted the need for renal replacement therapy after surgery. Most importantly, on top of a multivariable model of clinical parameters, the added value of either biomarker was limited. Future studies should focus on

the value of these biomarkers as part of a biomarker panel, which may more adequately predict AKI, or on their potential role in other populations at high risk of AKI.

Birth weight and kidney function

Finally, in middle-aged individuals of the general population, low birth weight has only a small effect on kidney function in middle aged individuals. It is plausible that in middle-aged individuals other risk factors or diseases during life have had more impact on kidney function than a person's birth weight. Low birth weight may be more important as a risk factor for CKD in younger patients. Given our results, low birth weight is at most weakly associated with kidney function at middle age, and as such may be irrelevant for risk stratification of middle-aged adults with regards to kidney disease.

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Chapter 10 -

Appendices

Dutch Summary - Nederlandse samenvatting
Dankwoord
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List of Publications

NEDERLANDSE SAMENVATTING

Inleiding

Gezonde nieren filteren afvalstoffen en schadelijke producten uit de bloedsomloop, maar reguleren ook de vochtbalans, de bloeddruk, aanmaak van rode bloedcellen, en de calcium-fosfaat huishouding. Een verstoorde werking van de nieren heeft dus grote gevolgen voor zowel onze lichamelijke als geestelijke gezondheid. De nierfunctie wordt bepaald door de snelheid waarmee de nierfilters, de glomeruli, het bloed filteren, en zo ontdoen van afvalstoffen. Deze glomerulaire filtratiesnelheid (glomerular filtration rate, GFR) wordt gemeten in milliliter per minuut, gecorrigeerd voor lichaamsoppervlakte. Met het ouder worden, neemt de nierfunctie af met ongeveer 1 mL/min/1,73m^2 per jaar boven de leeftijd van 40 jaar. Omdat deze natuurlijke daling van nierfunctie langzaam gaat, ontwikkelt de meerderheid van de mensen geen ernstige chronische nierschade (CNS) of nierfalen. Chronische nierschade wordt ingedeeld in stadia op basis van GFR (Tabel 1). Wel blijkt uit epidemiologisch onderzoek dat gemiddeld voor elke leeftijd een lagere nierfunctie gepaard gaat met een hoger risico op complicaties en overlijden, vergeleken met een hogere nierfunctie. Daarnaast hebben patiënten met bepaalde risicofactoren, zoals diabetes, hoge bloeddruk, of een ongezonde leefstijl, een snellere daling van de nierfunctie ten opzichte van gezonde leeftijdsgenoten, en daardoor een hoger risico op het ontwikkelen van chronische nierschade.

Tabel 1: Classificatie van Chronische Nierschade (CNS), gebaseerd op de glomerulaire filtratiesnelheid (GFR).

CNS stadium	GFR (mL/min/1,73m^2)	Terminologie
1	≥ 90	Normaal of hoog
2	60 to 89	Mild verminderd
3a	45 to 59	Mild tot matig verminderd
3b	30 to 44	Matig tot ernstig verminderd
4	15 to 29	Ernstig verminderd
5	< 15	Nierfalen

Wereldwijd is de prevalentie van chronische nierschade de afgelopen decaden sterk toegenomen, wat gepaard gaat met een toename van cardiovasculaire morbiditeit en mortaliteit, verlies van kwaliteit van leven, en substantiële kosten van de gezondheidszorg. In Europa heeft 11% van de bevolking van 45 jaar en ouder chronische nierschade (stadium 3, GFR <60 mL/min/1,73m^2). In 2016 overleden 1,2 miljoen mensen wereldwijd aan de gevolgen van

chronische nierschade. Daarmee staat nierziekte op de 12de plaats als oorzaak van overlijden, terwijl dit 20 jaar eerder nog plaats 27 was. De toename van chronische nierschade wordt grotendeels veroorzaakt door vergrijzing, ongezondere leefstijl, en de toename van cardiovasculaire risicofactoren zoals hoge bloeddruk en diabetes. Gezien de huidige trend van zowel vergrijzing als ongezonde leefstijl de komende jaren doorzet, zal het aantal patiënten met chronische nierschade ook blijven toenemen.

Diverse risicofactoren voor hart- en vaatziekte en leefstijl factoren dragen bij aan het risico op chronische nierschade. Voorbeelden van deze factoren zijn diabetes, hoge bloeddruk, roken, obesitas, ongezond dieet patroon, en gebrek aan lichamelijke beweging. De meeste factoren zijn met name onderzocht in gezonde populaties of in relatie tot hart- en vaatziekten. Er is echter nog weinig bekend over de rol van deze factoren in relatie tot de ontwikkeling van chronische nierschade in populaties met een verhoogd risico op hart- en vaatziekten zoals bij patiënten na een hartinfarct. Het feit dat juist deze hoog-risico patiënten een steeds grotere groep vormen, met name door toenemende vergrijzing en ongezonde leefstijl, benadrukt de noodzaak voor meer onderzoek toegespitst op deze patiënten. Meer kennis over potentieel modificeerbare risicofactoren, leidt mogelijk tot minder gebruik van farmacologische interventies. Dit proefschrift gaat vanuit epidemiologisch perspectief in op de rol van verschillende cardiovasculaire risicofactoren en leefstijl factoren bij de progressie van chronische nierschade. Hierbij ligt de focus met name, maar niet uitsluitend, op cardiovasculair hoog-risico groepen. Uiteindelijk leidt een toegenomen inzicht in de rol van verschillende (modificeerbare) factoren in progressie van nierschade mogelijk tot de ontwikkeling van behandelopties of richtlijnen, specifiek gericht op preventie en progressie van chronische nierschade.

Belangrijkste resultaten uit dit proefschrift per hoofdstuk

Hoofdstuk 2 tot en met 4 zijn uitgevoerd in het Alpha Omega Cohort, en hierin onderzochten we de rol van verschillende cardiovasculaire risicofactoren en leefstijl factoren bij de progressie van nierschade bij patiënten die een hartinfarct hadden doorgemaakt. Het gaat om een patiëntengroep van gemiddeld 69 jaar oud, 80% man, en medicamenteus behandeld volgens de medische richtlijnen. De mediane tijd na het hartinfarct was 4 jaar. De geschatte GFR (eGFR) was 81,5 mL/min/1,73m^2, gebaseerd op serum cystatine C. Initieel waren deze 4837 patiënten geïncludeerd in een gerandomiseerde interventie studie tussen 2002 en 2006, de Alpha Omega Trial, waarin het effect van suppletie van omega-3 vetzuren op onder andere recidief hartinfarct werd onderzocht. De Alpha Omega Trial is vervolgens voortgezet als cohort studie,

genaamd Alpha Omega Cohort. Voor de analyses in het huidige proefschrift werd ongeveer de helft van de 4837 patiënten geïncludeerd, namelijk alleen de patiënten van wie bloed afgenomen was bij inclusie en na 41 maanden follow-up. Dit waren de patiënten bij wie voor augustus 2009 de follow-up van 41 maanden compleet was. Omdat deze chronologische selectie gebaseerd was op financiële gronden, leidde dit niet tot selectiebias, maar hoogstens tot een lagere power. De onderzoeksvragen in de komende hoofdstukken zijn etiologisch van aard, wat inhoud dat de analyses zoveel mogelijk gecorrigeerd zijn voor factoren die de te onderzoeken verbanden kunnen verstoren (confounders).

In **Hoofdstuk 2** werd de associatie tussen diabetes, hoge bloeddruk (≥140/90 mmHg), hoog LDL-cholesterol (≥2,5 mmol/L), roken van sigaretten, en obesitas (body-mass index ≥30 kg/m^2) in relatie tot nierfunctie achteruitgang onderzocht. De gemiddelde nierfunctie daling van het cohort was 1,3 mL/min/1,73m^2 per jaar. Patiënten met diabetes of hoge bloeddruk, vergeleken met patiënten zonder deze risicofactoren, hadden een extra daling per jaar van 0,9 en 0,5 mL/min/1,73m^2, respectievelijk. Voor obesitas en roken was dit 0,3 en 0,2 mL/min/1,73m^2, en voor hoog LDL-cholesterol werd geen relatie gezien met een snellere daling van nierfunctie. Logistische regressie analyses toonden vergelijkbare resultaten: patiënten met diabetes of hoge bloeddruk, vergeleken met patiënten zonder deze factoren, hadden een 1,7 en 1,4 keer hoger risico op een jaarlijkse daling van tenminste 3 mL/min/1,73m^2. Vervolgens analyseerden we het verband tussen het hebben van meerdere risicofactoren en daling van nierfunctie, waarbij combinaties van diabetes, hoge bloeddruk, roken, en obesitas werden bestudeerd. Het bleek dat het hebben van meer van deze risicofactoren nauw samenhing met de snelheid waarmee de nierfunctie daalt, in deze hoog-risico patiënten. In patiënten die tenminste drie van deze vier factoren hadden, zagen we een gemiddelde jaarlijkse nierfunctie daling van 2,4 mL/min/1,73m^2, en een 2,6 keer hoger risico op een "snelle" daling van tenminste 3 mL/min/1,73m^2 per jaar, vergeleken met patiënten die geen van deze risicofactoren hebben. Patiënten zonder deze risicofactoren hadden een jaarlijkse daling van 0,9 mL/min/1,73m^2. Concluderend, deze resultaten suggereren dat het reduceren van risicofactoren, en optimaliseren van leefstijl, zinvol is in het kader van preventie van nierschade, in patiënten die een hartinfarct hebben door gemaakt.

In **Hoofdstuk 3** werd dieper ingegaan op obesitas als risicofactor voor progressie van chronische nierschade. De prevalentie van obesitas in de Westerse wereld neemt snel toe, hoofdzakelijk als gevolg van ongunstige leefstijlveranderingen. Hoewel obesitas het risico op chronische nierschade indirect verhoogt via verhoging van het risico op diabetes en hoge bloeddruk, zijn er ook directe mechanismen via welke obesitas nierschade kan induceren.

Obesitas creëert een staat van chronische inflammatie en leidt tot glomerulaire hyperfiltratie. Toch duiken er in de literatuur regelmatig publicaties op die concluderen dat obesitas beschermend zou zijn in chronisch zieke patiënten (de "obesitas paradox"). Mede om deze reden, werd in dit proefschrift een separaat hoofdstuk aan dit vraagstuk gewijd. In patiënten die een hartinfarct hadden meegemaakt werd onderzocht wat de associatie tussen overgewicht en nierfunctie daling was, waarbij body-mass index (BMI) en middelomtrek als maten van overgewicht gebruikt werden. De middelomtrek wordt gezien als een meer representatieve maat voor visceraal vet. Analyses in categorieën van BMI, toonden dat met name patiënten met een BMI van meer dan 30 kg/m^2 (obesitas) een snellere daling hadden dan patiënten met een BMI van minder dan 25 kg/m^2. Wanneer BMI niet in categorieën, maar als continue maat, werd geanalyseerd, was iedere 5 kg/m^2 BMI geassocieerd met een extra jaarlijkse nierfunctie daling van 0,21 (95% betrouwbaarheids-interval: 0,10; 0,46) mL/min/1,73m^2. We vonden vergelijkbare verbanden voor mannen en vrouwen, en vergelijkbare uitkomsten voor BMI en middelomtrek. We concludeerden dat in stabiele post-hartinfarct patiënten een hogere BMI of middelomtrek geassocieerd was met een snellere daling van nierfunctie. Deze conclusie is in lijn met de huidige richtlijnen, die adviseren naar een BMI van minder dan 25 kg/m^2 te streven. Onze resultaten pleiten tegen het bestaan van een "obesitas paradox".

In **Hoofdstuk 4** stond de dagelijkse inname van eiwit uit voeding centraal. In de huidige nefrologische richtlijnen wordt voor bepaalde patiënten groepen met hoog risico op chronische nierschade een eiwitbeperkt dieet geadviseerd namelijk <0,8 gram per kg lichaamsgewicht per dag. Echter, voor gezonde personen, of hoog-risico patiënten met nog een relatief goede nierfunctie, bestaan nog geen heldere adviezen. In dit hoofdstuk werd daarom het verband tussen eiwit inname uit voeding en daling van nierfunctie onderzocht, in post-hartinfarct infarct patiënten met een relatief goede nierfunctie. Omdat er aanwijzingen zijn dat eiwit uit plantaardige bron gezonder zou zijn dan uit dierlijke bron, onderzochten we tevens de associaties van eiwit uit deze verschillende bronnen apart. Uit de analyses bleek een duidelijk lineair verband tussen een hogere totale eiwit inname en snellere nierfunctie daling. Patiënten met een dagelijkse eiwit inname van meer dan 1,2 g/kg lichaamsgewicht vergeleken met <0,8 g/kg, hadden een twee keer snellere jaarlijkse nierfunctie daling (1,60 vergeleken met 0,84 mL/min/1,73m^2). Met additionele spline analyses, waarbij verbanden flexibeler gemodelleerd worden, werd dit sterke lineaire verband bevestigd. Het verband tussen nierfunctie en plantaardig of dierlijk eiwit was vergelijkbaar. Ook werd geen verschil in effect gevonden voor mannen en vrouwen. Concluderend, post-hartinfarct patiënten met een lagere

eiwit inname hadden een minder snelle afname van nierfunctie. Dit impliceert dat in deze post-hartinfarct patiënten, met een relatief goede nierfunctie, een eiwitbeperkt dieet een reële interventie kan zijn bij de preventie van progressie van chronische nierschade.

Hoofdstuk 5 richtte zich op type 1 diabetes patiënten met eind-stadium nierfalen. Type 1 diabetes wordt veroorzaakt door een auto-immuunreactie tegen de insuline-producerende β-cellen van de pancreas, en vormt 5-10% van het wereldwijde aantal patiënten met diabetes. Type 1 diabetes ontwikkelt zich meestal op kinderleeftijd of in tijdens de adolescentie, en leidt tot een 7% cumulatief risico op het ontwikkelen van eind-stadium nierfalen binnen 30 jaar. Door dit hoge risico op nierfalen, zal een substantieel deel van de type 1 diabetes patiënten uiteindelijk in aanmerking komen voor niertransplantatie. Echter, niertransplantatie vormt geen behandeling van de diabetes. Het transplanteren van zowel een nier als een pancreas verbetert zowel de nierfunctie als de diabetes, maar het transplanteren van twee organen geeft ook een hoger risico op korte-termijn complicaties zoals mortaliteit en rejectie. Bovendien tonen de studies tot nu toe niet eenduidig aan dat een gecombineerde nier-pancreas transplantatie substantieel beter is dan een niertransplantatie alleen, en beperkt voorgaand onderzoek zich vaak tot een follow-up tijd ruim onder de 10 jaar. Wij hebben daarom onderzocht of een simultane nier-pancreas transplantatie geassocieerd was met een betere patiënt overleving, ten opzichte van een niertransplantatie alleen van zowel een overleden als levende donor. Hiervoor hebben we gebruik gemaakt van registratie data van alle 2796 type 1 diabetes patiënten die getransplanteerd zijn in Nederland in de periode 1986 – 2016. Daarnaast maakt de lange follow-up van onze data ook onderzoek naar uitkomsten op de lange termijn, 10 en 20 jaar na transplantatie, mogelijk.

Na correctie voor confounders, bleken patiënten met een gecombineerde nier-pancreas transplantatie respectievelijk een 33% en 15% lagere kans te hebben om binnen 10 jaar te overlijden, ten opzichte van patiënten die alleen een nier ontvingen van een levende of overleden donor. De hoogste overleving werd geobserveerd voor nier-pancreas patiënten waarbij de getransplanteerde pancreas na één jaar nog functioneerde. Echter, de patiënten die de verschillende typen transplantaties ondergaan verschillen intrinsiek van elkaar, zowel qua pre-transplantatie traject, donor en ontvanger karakteristieken. Ondanks correctie voor dergelijke factoren, blijft een directe vergelijking tussen verschillende transplantatie groepen lastig. Daarom hebben we gebruik gemaakt van het feit dat in Nederland de verschillende transplantatie centra een voorkeur hebben voor ofwel het transplanteren van een nier met pancreas (regio Leiden), ofwel het transplanteren van een nier alleen (overige regio's). Op deze manier werd de invloed van verschillen in patiënt karakteristieken tussen

transplantatie typen op de uitkomst gereduceerd. Met deze analyse toonden we aan dat een behandelstrategie met een voorkeur voor een gecombineerde nier-pancreas transplantatie geassocieerd was met een 44% lagere 10-jaars mortaliteit dan een strategie met een voorkeur voor een niertransplantatie alleen. Hieruit concluderen we dat voor type 1 diabetes patiënten met eindstadium nierfalen, het transplanteren van een nier plus pancreas waarschijnlijk de voorkeur verdient boven het transplanteren van een nier alleen.

Hoofdstuk 6 is een netwerk meta-analyse waar de potentiële gunstige effecten van verschillende statines ten aanzien van preventie van chronische nierschade in kaart gebracht worden. We includeerden alle gerandomiseerde interventie studies, met ten minste één jaar follow-up, waarin het effect van een statine op de nierfunctie of het optreden van proteïnurie werd gerapporteerd. Dit resulteerde in 43 studies met in totaal meer dan 110.000 patiënten, die tezamen zeven verschillende statines onderzochten. Wanneer alle statines samen vergeleken werden met controle of placebo patiënten, bleken statines een voordelig effect te hebben op de nierfunctie. Gebruik van statines resulteerde in een nierfunctie daling die 0,5 mL/min/1,73m^2 per jaar trager was dan in de controle groep. Ook werd een klein gunstig effect op progressie van proteïnurie aangetoond, hoewel de data in dit geval suggestief waren voor mogelijke publicatiebias. In de uiteindelijke netwerk meta-analyse bleek dat over het algemeen iedere statine een voordelig effect had op beide eindpunten, vergeleken met de controle interventie. Echter, voor vergelijkingen tussen individuele statines bestond veel overlap tussen de betrouwbaarheidsintervallen, waardoor het niet mogelijk was om van één bepaalde statine superioriteit te concluderen. Concluderend, gebruik van statines vergeleken met placebo, leidde tot een tragere achteruitgang van nierfunctie, en vertraagde mogelijk ook de progressie van proteïnurie. Of één bepaalde statine de voorkeur verdient in het kader van progressie van chronische nierschade kan aan de hand van de huidige studie niet vastgesteld worden.

In **Hoofdstuk 7** werd de waarde van twee urine biomarkers voor het voorspellen van acute nierschade onderzocht, in electieve cardiochirurgie patiënten. Acute nierschade is een plotselinge episode van nierfunctie daling, gepaard gaande met een stijging van serum kreatinine waarden en verlaagde urine productie. Vaak is acute nierschade het gevolg van medisch handelen, bijvoorbeeld door peri-operatief verlaagde doorbloeding van de nier. Acute nierschade gaat gepaard met verhoogde mortaliteit en verhoogd tevens het risico op chronische nierziekte. Omdat acute nierschade moeilijk te voorspellen is, en het serum creatinine pas in een relatief laat stadium stijgt, wordt de diagnose acute nierschade vaak te laat gesteld, namelijk als irreversibele schade al is opgetreden. Er is daarom behoefte aan nieuwe biomarkers die acute

nierschade in een vroeg stadium al kunnen voorspellen of diagnosticeren. Twee potentiële kandidaten zijn tissue inhibitor of metalloproteinases 2 (TIMP-2) en Insulin-like growth factor-binding protein 7 (IGFBP7), beide reeds goedgekeurd door de Food and Drug Administration in de Verenigde Staten in het kader van acute nierschade. Eerder gepubliceerde resultaten van de voorspellende waarde van beide biomarkers waren veelbelovend. Wij hebben onderzocht of deze biomarkers toegevoegde waarde hadden om in een vroeg stadium ernstige acute nierschade te voorspellen, waarbij nierfunctie vervangende therapie noodzakelijk was, in een cohort van patiënten die electieve cardiochirurgie ondergingen tussen 2006 en 2010. De discriminatieve waarde van beide biomarkers met betrekking tot het voorspellen van noodzaak voor nierfunctie vervangende therapie bleek redelijk. Met name IGFBP7 waarden voorafgaand aan de operatie, en de verandering van TIMP-2 waarden gedurende de operatie, hadden een goede discriminatieve waarde. Toevoeging van deze biomarkers in een predictiemodel met basale klinische variabelen leidde tot een beperkte verbetering van de reeds zeer goede voorspellende waarde van deze modellen. Wij concludeerden daarom dat deze biomarkers redelijk presteerden in het voorspellen van acute nierschade in deze patiëntengroep. De klinische relevantie met name ook bij minder ernstige vormen van acute nierschade blijft nog onduidelijk.

In **Hoofdstuk 8** onderzochten we de associatie tussen geboortegewicht en nierfunctie op middelbare leeftijd (45 tot 65 jaar) van gezonde proefpersonen van de Netherlands Epidemiology of Obesity (NEO) studie. De NEO studie is opgezet om de invloed van obesitas op verschillende chronische ziekten te onderzoeken. De studie wordt gekenmerkt door een overrepresentatie van individuen met een BMI boven de 27 kg/m^2. Door de analyses te herwegen naar de BMI verdeling van de algemene Nederlandse populatie, zijn de resultaten toch te generaliseren naar de algemene populatie. In dit hoofdstuk is de relatie tussen geboortegewicht en nierfunctie op middelbare leeftijd op drie manieren onderzocht. In de NEO studie is geboortegewicht van de deelnemers door middel van vragenlijsten geregistreerd. Na correctie voor confounders werd geen verband gevonden tussen geboortegewicht en nierfunctie. Vervolgens werd een Mendeliaanse randomisatie analyse uitgevoerd, waarbij als instrument voor geboortegewicht een gewogen genetische score gebruikt wordt, die bestond uit 59 genetische varianten die met geboortegewicht samenhingen. Deze genetische varianten zijn eerder gepubliceerd in een "genome wide association study", en verklaarden ongeveer 2% van de variatie in geboortegewicht. Hoewel 2% laag lijkt, is dit voor Mendeliaanse randomisatie studies niet ongebruikelijk. De genetische score voor geboortegewicht was in de NEO deelnemers geassocieerd met het gerapporteerde geboortegewicht, en voldeed aan de eisen

voor een valide instrumentele variabele. Voorts werd een statistisch significante relatie tussen de genetische score en nierfunctie aangetoond in de NEO studie. Een 500-gram genetisch verhoogd geboortegewicht was geassocieerd met een 3,7 mL/min/1,73m^2 hogere nierfunctie op middelbare leeftijd. Tenslotte werd ter validatie een "two-sample" Mendeliaanse randomisatie studie uitgevoerd, met dezelfde genetische score voor geboortegewicht als determinant. Hier werd gebruik gemaakt van publiekelijk beschikbare summary-data van de genetische nierfunctie data van 133.814 individuen van het CKDgen consortium. De resultaten toonden geen effect van geboortegewicht op nierfunctie op middelbare leeftijd. Concluderend, op basis van deze resultaten was er geen eenduidig effect van geboortegewicht op nierfunctie op middelbare leeftijd.

Conclusies en implicaties

In dit proefschrift hebben we aangetoond dat niet alle post-hartinfarct patiënten een versnelde nierfunctiedaling hebben, vergeleken met de gemiddelde jaarlijkse daling in de algemene bevolking. Zowel het aantal risicofactoren dat een patiënt heeft, als het type risico factor, bepaalt de snelheid van de nierfunctiedaling. Hoe meer risicofactoren een patiënt heeft, hoe sneller de nierfunctiedaling. Diabetes en hoge bloeddruk zijn de risicofactoren met het sterkste effect op nierfunctiedaling. Het optimaliseren van cardiovasculaire risicofactoren is dus van belang, naast de standaard medicamenteuze behandeling, om progressie van chronische nierschade te vertragen. Daarnaast toonden we aan dat obesitas bij post-hartinfarct patiënten een nadelig effect heeft op de snelheid van nierfunctiedaling. Deze resultaten zijn in lijn met de huidige Kidney Disease Improving Global Outcomes (KDIGO) richtlijnen, welke adviseren te streven naar een gezond gewicht wat overeenkomt met een body-mass index lager dan 25 kg/m^2. In het kader van cardiovasculair risico management, concluderen we ook dat het gebruik van een statine, onafhankelijk van de cholesterol waarden, gepaard gaat met een vertraagde nierfunctiedaling. Echter, we konden niet aantonen dat één specifieke statine het meest effectief was, ter voorkoming of vertraging van progressie van chronische nierschade. Ook deze resultaten onderstrepen de huidige KDIGO richtlijnen, die adviseren onafhankelijk van cholesterol waarden een statine voor te schrijven aan niet-dialyse afhankelijke chronische nierschade patiënten van 50 jaar of ouder, met een nierfunctie van minder dan 60 mL/min/1,73m^2, of ten minste 30 mg/g albuminurie. De richtlijnen specificeren niet welke statine de voorkeur verdient.

Naast de welbekende cardiovasculaire risicofactoren, kunnen ook aanpassingen aan het dieet van invloed zijn op progressie van chronische nierschade. Ons observationele onderzoek toont aan dat een eiwit-beperkt dieet waardevol kan zijn bij het vertragen van progressie van chronische nierschade.

Echter, gerandomiseerde studies naar de effecten van een eiwit-beperkt dieet zijn geïndiceerd om uiteindelijk onomstotelijk bewijs te leveren voor het gunstige effect van eiwitbeperkt dieet op progressie van chronische nierschade.

Op basis van data van alle type 1 diabetes mellitus patiënten in Nederland met nierfunctie vervangende therapie tussen 1986 en 2016, bleek dat behandeling met gecombineerde nier-pancreas transplantatie vergeleken met een nier transplantatie alleen, gepaard ging met een betere overleving. Voor deze patiëntengroep zou daarom mogelijk de eerste keuze van behandeling een simultane nier-pancreas transplantatie moeten zijn.

In electieve cardiochirurgie patiënten hebben de urine biomarkers TIMP-2 en IGFBP7 mogelijk een toegevoegde waarde voor het voorspellen van acute nierschade in een vroeg stadium. Hoewel beide biomarkers in andere heterogene intensive care populaties zeer goede voorspellers voor acute nierschade bleken te zijn, was de waarde in de relatief gezonde populatie die wij onderzochten beperkter. De focus van toekomstige studies in electieve cardiochirurgie patiënten zou moeten liggen bij het onderzoeken van de waarde van deze biomarkers als deel van een biomarker panel.

Tenslotte, een laag geboortegewicht bleek marginaal geassocieerd met een verminderde nierfunctie op middelbare leeftijd. Op middelbare leeftijd is waarschijnlijk het effect van cardiovasculaire risicofactoren en ongezonde leefstijl op progressie van chronische nierschade groter dan een laag geboorte gewicht.

DANKWOORD

Graag wil ik eenieder die dit proefschrift mede mogelijk gemaakt heeft, bedanken.

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Ook ben ik de promovendi, stafleden, en leden van het secretariaat van de afdelingen Nefrologie en Klinische Epidemiologie dankbaar. Door de gezellige en collegiale werksfeer en gezamenlijke lunches heb ik het op beide afdelingen altijd erg naar mijn zin gehad.

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CURRICULUM VITAE

Kevin Esmeijer werd geboren op 2 maart 1990, te 's Gravenhage. In 2008 behaalde hij zijn gymnasium diploma cum laude, aan het Veurs Lyceum te Leidschendam, waarna hij de opleiding Biomedische Wetenschappen begon aan de Universiteit Leiden. Na in 2011 de Bachelor behaald te hebben, werd hij aangenomen bij het zogeheten "dubbel-traject". Dit traject, aangeboden in het Leids Universitair Medisch Centrum, bood de mogelijkheid om zowel de studie Biomedische Wetenschappen als Geneeskunde parallel te volgen en af te ronden. Tijdens zijn studie heeft hij onder andere het Biomedische Wetenschappen symposium mede-georganiseerd, en heeft hij enkele maanden in Heidelberg, Duitsland, gestudeerd. Zijn interesse voor met name beschouwende medische vakken blijkt uit de wetenschappelijke stages op de afdelingen Nierziekten en Klinische Epidemiologie in het Leids Universitair Medisch Centrum, en de gevolgde semi-artsstage Neurologie in het Haga ziekenhuis, te 's Gravenhage. Na acht studiejaren behaalde hij in augustus 2016 zowel zijn Master diploma voor Biomedische Wetenschappen (cum laude) als Geneeskunde.

Aansluitend startte hij in september 2016 zijn promotietraject onder supervisie van Prof. Dr. Johan de Fijter, Prof. Dr. Frits Rosendaal en Dr. Ellen Hoogeveen, op de afdeling Nierziekten in het Leids Universitair Medisch Centrum, in samenwerking met het Jeroen Bosch ziekenhuis. Het promotietraject vloeide voort uit de tweede wetenschappelijke stage van de Master Biomedische Wetenschappen, en behelsde het in kaart brengen van modificeerbare risicofactoren voor nierfunctie daling bij cardiovasculair belaste patiënten. Gedurende de promotie periode van tweeënhalf jaar heeft hij veel epidemiologisch onderwijs gegeven aan studenten Geneeskunde en Biomedische Wetenschappen, op enkele nationale en Europese congressen resultaten van wetenschappelijk onderzoek gepresenteerd, een zevental wetenschappelijke artikelen geschreven, en een groot aantal epidemiologische cursussen gevolgd, resulterend in het behalen van de titel Epidemioloog B. In maart 2019 is hij gaan werken als arts-assistent op de afdeling Interne Geneeskunde van het Haaglanden Medisch Centrum, en sinds 1 januari 2020 is hij in ditzelfde ziekenhuis gestart met de opleiding tot internist.

Tenslotte is hij in mei 2019 vader geworden van een dochter, Lune, en verloofd met zijn partner, Cynthia.

LIST OF PUBLICATIONS

1. **Esmeijer K**, Geleijnse JM, de Fijter JW, Giltay EJ, Kromhout D, Hoogeveen EK. Cardiovascular Risk Factors Accelerate Kidney Function Decline in Post-Myocardial Infarction Patients: The Alpha Omega Cohort Study. *Kidney Int Rep.* 2018; 3: 879-888.

2. **Esmeijer K**, Geleijnse JM, Giltay EJ, Stijnen T, Dekker FW, de Fijter JW, Kromhout D, Hoogeveen EK. Body-fat indicators and kidney function decline in older post-myocardial infarction patients: The Alpha Omega Cohort Study. *Eur J Prev Cardiol.* 2018; 25: 90-99.

3. **Esmeijer K**, Geleijnse JM, de Fijter JW, Kromhout D, Hoogeveen EK. Dietary protein intake and kidney function decline after myocardial infarction: the Alpha Omega Cohort. *Nephrol Dial Transplan*t. 2020; 35: 106-115.

4. **Esmeijer K,** de Vries AP, Mook-Kanamori DO, de Fijter JW, Rosendaal FR, Rabelink TJ, Smit RAJ, de Mutsert R, Hoogeveen EK. Low Birth Weight and Kidney Function in Middle-Aged Men and Women: The Netherlands Epidemiology of Obesity Study. *Am J Kidney Dis.* 2019; 74: 751-760.

5. **Esmijer K,** Dekkers OM, de Fijter JW, Dekker FW, Hoogeveen EK. Effect of different types of statins on kidney function decline and proteinuria: a network meta-analysis. *Sci Rep.* 2019; 9: 16632

6. **Esmeijer K**, Hoogeveen EK, van den Boog PJM, Konijn C, Mallat MJK, Baranski AG, Dekkers OM, de Fijter JW. Superior Long-Term Survival for Simultaneous Pancreas-Kidney Transplantation as Renal Replacement Therapy: 30-Year Follow-up of a Nationwide Cohort. *Diabetes Care*. 2020; 43: 321-328.

7. **Esmeijer K**, Schoe A, Ruhaak LR, Hoogeveen EK, Soonawala D, Romijn FPHTM, Shirzada MR, van Dissel JT, Cobbaert CM, de Fijter JW. The predictive value of TIMP-2 and IGFBP7 for kidney failure and 30-day mortality after elective cardiac surgery. *Submitted.*