Risk factors of chronic kidney disease progression: Dutch cohort studies
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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.

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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 $eGFR_{cr\text{-}cysC}$ 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 $eGFR_{cr\text{-}cysC}$ (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 $eGFR_{cr\text{-}cysC}$, 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 $eGFR_{cr\text{-}cysC}$ 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.

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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/1.73m²)	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

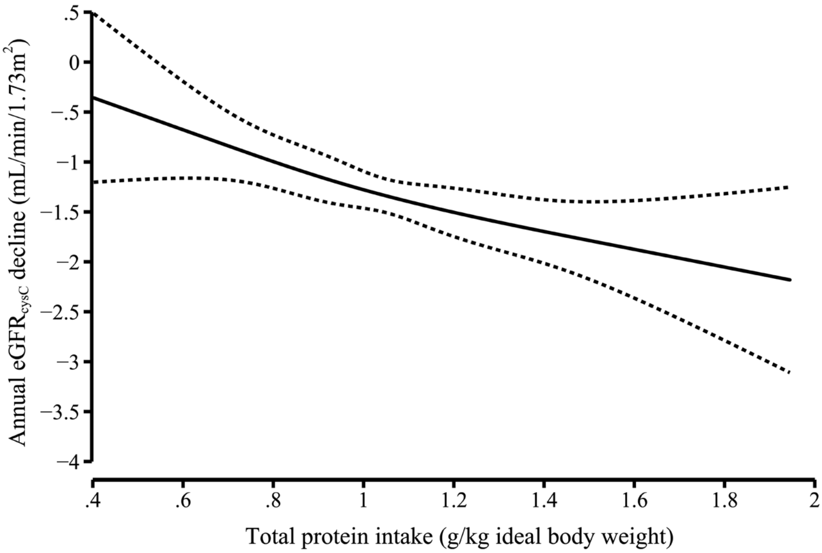
$eGFR_{cysC}$, cystatin C based estimated glomerular filtration rate
Model 1: adjusted for age, sex, and total energy intake.
Model 2: Model 1 plus additional adjustment for education, alcohol, smoking, physical activity, RAS blocking drugs.
Model 3: Model 2 plus additional adjustment for intake of fat (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.

A.



B.

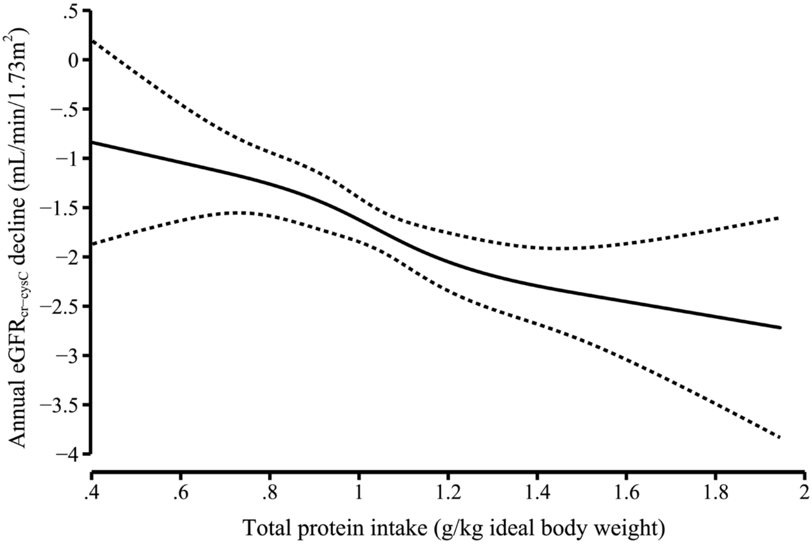


Figure 1: Association (with 95%-confidence interval) between daily total protein intake (g/kg ideal body weight) and annual cystatin C based (A) and creatinine-cystatin C based (B) eGFR. Modelled by restricted cubic splines with knots at the 5th, 35th, 65th, and 95th percentile of protein intake. In 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.

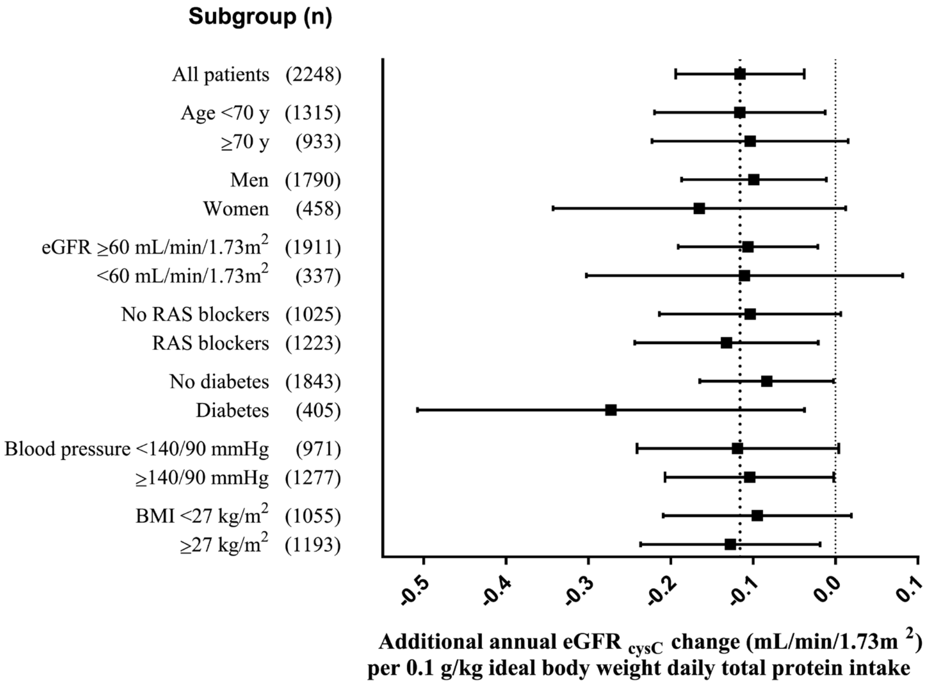


Figure 2: Additional annual change in eGFR$_{cysC}$ per 0.1 g/kg ideal body weight increased daily total protein intake, according to different subgroups. The model was fully adjusted (model 3) for age, sex, total energy intake, education, alcohol, smoking, physical activity, RAS blocking drugs, for 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)				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 $eGFR_{cr-cysC}$ 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/1.73m²)	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

$eGFR_{cr-cysC}$, 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.

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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.

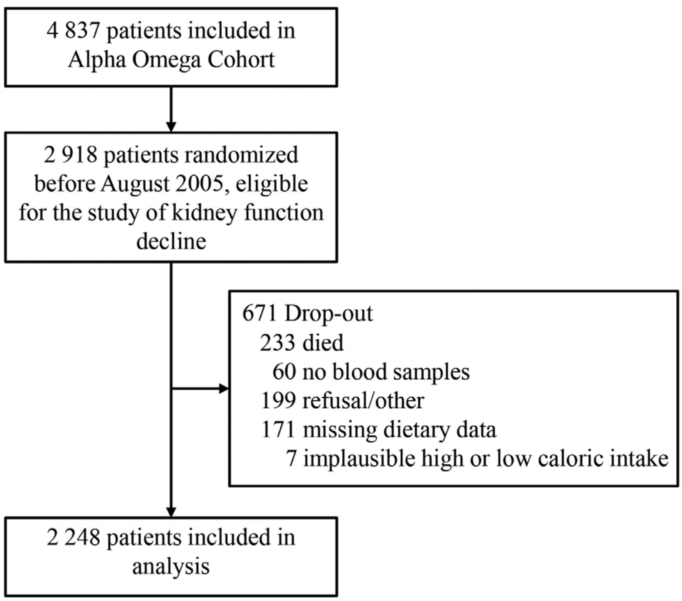
Unit		Total protein	Animal protein			Plant protein
			Total	From meat	From dairy	
0.1 g/kg ideal	Crude	−0.02 (−0.07; 0.04)	−0.05 (−0.12; 0.02)	−0.07 (−0.18; 0.05)	−0.05 (−0.15; 0.5)	0.10 (−0.03; 0.24)
body weight	Model 1	−0.11 (−0.19; −0.03)*	−0.12 (−0.20; −0.04)*	−0.11 (−0.23; 0.01)	−0.10 (−0.21; 0.1)	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.