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Title page

Dietary n-3 Polyunsaturated Fatty Acid Intake and All-cause and Cardiovascular Mortality in Adults on Hemodialysis: The DIET-HD Multinational Cohort Study

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Running Title: Long chain n-3 PUFA dietary intake and mortality in HD

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Abstract

Background & Aims: Patients on hemodialysis suffer from high risk of premature death, which is largely attributed to cardiovascular disease, but interventions targeting traditional cardiovascular risk factors have made little or no difference. Long chain n-3 polyunsaturated fatty acids (n-3 PUFA) are putative candidates to reduce cardiovascular disease. Diets rich in n-3 PUFA are recommended in the general population, although their role in the hemodialysis setting is uncertain. We evaluated the association between the dietary intake of n-3 PUFA and mortality for hemodialysis patients.

Methods: The DIET-HD study is a prospective cohort study (January 2014-June 2017) in 9757 adults treated with hemodialysis in Europe and South America. Dietary n-3 PUFA intake was measured at baseline using the GA^{2}LEN Food Frequency Questionnaire. Adjusted Cox regression analyses clustered by country were conducted to evaluate the association of dietary n-3 PUFA intake with cardiovascular and all-cause mortality.

Results: During a median follow up of 2.7 years (18,666 person-years), 2087 deaths were recorded, including 829 attributable to cardiovascular causes. One third of the study participants consumed sufficient (at least 1.75 g/week) n-3 PUFA recommended for primary cardiovascular prevention, and less than 10% recommended for secondary prevention (7-14 g/week). Compared to patients with the lowest tertile of dietary n-3 PUFA intake (<0.37 g/week), the adjusted hazard ratios (95% confidence interval) for cardiovascular mortality for patients in the middle (0.37 to <1.8 g/week) and highest (≥1.8 g/week) tertiles of n-3 PUFA were 0.82 (0.69-0.98) and 1.03 (0.84-1.26), respectively. Corresponding adjusted hazard ratios for all-cause mortality were 0.96 (0.86-1.08) and 1.00 (0.88-1.13), respectively.

Conclusions: Dietary n-3 PUFA intake was not associated with cardiovascular or all-cause mortality in patients on hemodialysis. As dietary n-3 PUFA intake was low, the possibility that n-3 PUFA supplementation might mitigate cardiovascular risk has not been excluded.

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Key words: n-3 PUFA, omega 3, fish oil, end stage renal disease, hemodialysis, mortality

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Abbreviations

DIET-HD study:	"DIETary intake, death and hospitalization in adult with end-stage kidney disease treated with HemoDialysis" study
n-3 PUFA	Long chain n-3 polyunsaturated fatty acids
GA^{2}LEN FFQ:	Global Allergy and Asthma European Network Food Frequency Questionnaire
CI	Confidence interval

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Introduction

Approximately 1 in 10 people on dialysis die every year, and 40% of these deaths are attributable to cardiovascular disease (1-3). The pathogenesis of cardiovascular disease is different in dialysis patients from for the general population, driven largely by non-traditional risk factors, including oxidative stress, inflammation, endothelium dysfunction and altered mineral metabolism leading to medial arterial calcification (4-7). Consequently, the benefits of interventions targeting traditional cardiovascular risk factors, such as statins, blood-pressure lowering, and anti-platelet therapy, have been shown to have lower effectiveness for preventing adverse cardiovascular outcomes in hemodialysis patients (8-12).

Long chain n-3 polyunsaturated fatty acids (n-3 PUFA) are recommended to prevent cardiovascular disease in the general population (13-15). n-3 PUFA, present mostly in oily fish and in smaller amount in meat, eggs and dairy products, have potential anti-thrombotic, anti-oxidative, anti-inflammatory and anti-arrhythmic effects on cardiac myocytes (16-19). Informed by a number of randomized controlled trials and systematic reviews (20-25), the current recommendations by the American Heart Association and World Health Organization suggest an intake of at least 1.75 grams per week of n-3 PUFA (achieved by at least two servings of fish per week, especially oily fish) for primary cardiovascular prevention and 7-14 grams per week (which could require supplementation) for secondary prevention (13-15).

In patients on hemodialysis, data for the effects of n-3 PUFA on mortality are sparse and limited to small-scale observational studies (26-33). Results to date have been inconclusive. Accordingly, recommendations for n-3 PUFA intake in the hemodialysis population have been extrapolated from evidence in the general population (34).

The aim of this study was to ascertain the association of dietary n-3 PUFA intake with cardiovascular and all-cause mortality among adult patients on hemodialysis (35).

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Materials and methods

Study Design

The "DIETary intake, death and hospitalization in adult with end-stage kidney disease treated with HemoDialysis" (DIET-HD) study is a multinational, prospective, cohort study to evaluate the association between nutrition and dietary patterns with major health outcomes in prevalent adult patients treated with hemodialysis. The study protocol has been detailed elsewhere (35). This study is reported according to the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines (36).

Study Population

Consecutive patients were invited from a convenience sample of clinics within a private dialysis provider network in Europe (France, Germany, Italy, Hungary, Poland, Portugal, Romania, Spain, Sweden, and Turkey) and South America (Argentina). Eligible patients were 18 years or older with end-stage kidney disease treated with hemodialysis (any number of treatments per week and any duration per treatment) for at least the previous 90 days. Patients were excluded if they had significant neurocognitive disability that precluded them from completing the Food Frequency Questionnaire (FFQ), a life expectancy less than six months, or anticipated kidney transplantation within six months of baseline data collection. Ethics approval was obtained from all relevant institutional ethics committees and the study was conducted in accordance with the Declaration of Helsinki. All participants provided written and informed consent.

Covariates of interests

Socio-demographic, clinical and dialysis characteristics at baseline were obtained from an administrative database that stored relevant data on incident patients requiring hemodialysis within all facilities of the private dialysis provider. This database was linked to the participants via a unique identification code. All participating clinics used the same standard operating procedures to

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assess and record baseline variables including age, gender, country of treatment, education attainment, marital status and living situation, occupation, smoking history, physical activity, Body Mass Index,comorbidities (including diabetes and previous cardiovascular disease), use of medications, laboratory parameters (including hemoglobin, albumin, phosphorus and calcium) and dialysis-related data(including time on dialysis and Kt/V).

Exposures

The dietary intake of n-3 PUFA was ascertained using the Global Allergy and Asthma European Network (GA^{2}LEN) Food Frequency Questionnaire (FFQ) (37). During the dialysis treatment, participants answered the FFQ, either independently or assisted by an interviewer, depending on the severity of their clinical condition. Data from the FFQ were entered into an electronic database using optical character recognition and linked to the baseline and outcomes data via a unique identification code. The GA^{2}LEN FFQ was specifically designed as the first single, standardized instrument to assess dietary intake across countries and was particularly validated for n-3 PUFA dietary intake. Patients reported how often they had consumed the foods over the previous year, using eight predefined options (rarely or never, 1-3 times per month, once, 2-4, or 5-6 times per week, once, 2-3 or 4 or more times per day). Standard food portion sizes were used to quantify the intake following the recommendations from the UK's Food Standards Agency, and daily food intake (grams) was calculated. Macro- and micronutrient intake were derived using the latest available McCance & Widdowson's Food Composition Tables.

Outcomes

The primary outcome was time to death due to cardiovascular causes. Cardiovascular mortality was defined as sudden death or death attributed to acute myocardial infarction, pericarditis, atherosclerotic heart disease, cardiomyopathy, cardiac arrhythmia, cardiac arrest, valvular heart disease, pulmonary edema, or congestive cardiac failure. Outcomes data were obtained from data

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linkage with the administrative database and adjudicated by the participants' treating clinicians, who were unaware of the dietary n-3 PUFA intake. The secondary outcome was death from any cause.

Statistical analysis

The *a priori* sample size calculation has been reported in detail in the published study protocol (35). Participants were excluded if their FFQ contained erroneous or missing identification code (after optical character recognition) that prevented the data linkage with their clinical baseline and outcomes data, 20% or more missing answers, or biologically implausible values for total energy intake (above or below 3 standard deviations from the log transformed mean).

Baseline variables were calculated as mean and standard deviation or median and interquartile range for continuous variables, depending upon their distribution, and as frequencies and percentages for categorical variables. Restricted cubic splines were used to determine the linearity between n-3 PUFA intake and mortality (no evidence of non-linearity was identified). The follow-up period was defined from the time of the inclusion in the study to the time of cardiovascular or all-cause mortality. Patients who left the dialysis network, underwent kidney transplantation, were transferred to peritoneal dialysis, withdrew dialysis, had kidney function recovery, went on vacation, were lost to follow-up or survived until the end of the follow-up period were censored.

Univariate and multivariate Cox proportional hazard regression analyses were fitted using a random effects shared frailty model and stratified by country to account for clustering of mortality risk and dietary exposure within countries. Dietary intake of n-3 PUFA was entered as tertiles in the random effect shared frailty model and as continuous variable in the analysis stratified by country. Results were expressed as a hazard ratio and the associated 95% confidence interval. The proportional hazards assumption in Cox models was assessed by fitting log (time)-dependent covariates in the multivariable model and no deviation from the assumption was found. Effect modification between

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dietary n-3 PUFA intake and covariates were tested in the multivariable model and no effect modification was observed.

Analyses of cardiovascular mortality were adjusted for gender, education (secondary versus none/primary), smoke (former or current versus never), diabetes, myocardial infarction, vascular access type (arterio-venous fistula versus graft/catheter), Body Mass Index (categories according to WHO), albumin (tertiles), Charlson comorbidity score (quartiles), age (standard deviation increase), phosphorus, calcium, haemoglobin, KTV (index to quantify hemodialysis treatment adequacy), fiber daily intake (tertiles) and energy intake (1000 kcal per day increase). Analyses of all-cause mortality were adjusted as above except for fiber intake and with the addition of time on dialysis and being wait-listed for renal transplantation. Variables included in the multivariate models were selected by backwards elimination retaining those (a part from energy intake and gender) that were significantly associated with mortality ($p < 0.05$) or changed the hazard ratio of mortality for dietary n-3 PUFA by a clinically relevant amount ($\geq 10\%$). For each categorical variable, an extra category was included for missing data in the multivariate model, when necessary (education, smoking, diabetes, myocardial infarction, serum albumin, Body Mass Index). A complete-case analysis was also conducted including only those patients with complete data as sensitivity analyses. In subgroup analysis the association between dietary n-3 PUFA intake and cardiovascular mortality was assessed among patients achieving levels of n-3 PUFA recommended for secondary cardiovascular prevention. The potential relevance of competing events (death from other causes and kidney transplantation for the analysis of cardiovascular mortality; kidney transplantation for the analysis of all-cause mortality) was considered using a stratified proportional sub-distribution hazard model. All analyses were conducted using SAS 9.4. (Inc, Gary 2014) and STATA version 14. A two tailed $P < 0.05$ was considered to indicate statistical significance.

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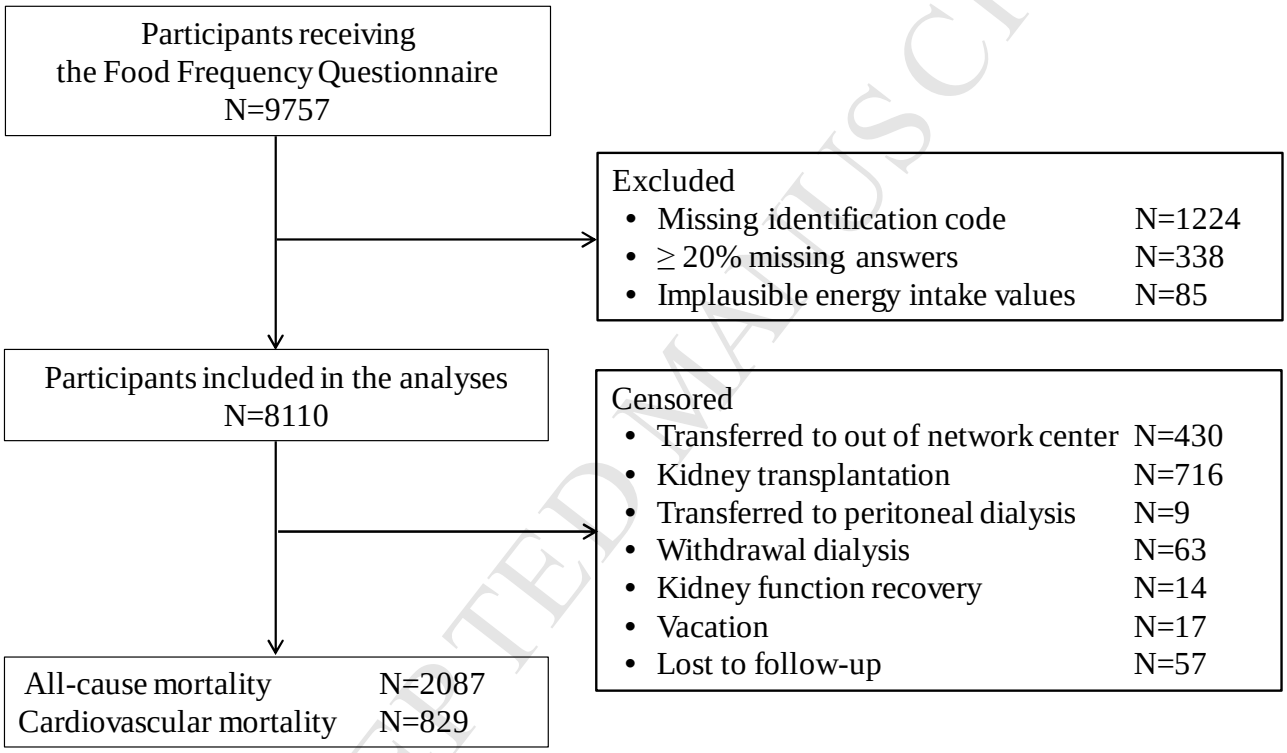
Results

Overall, 9757 patients on hemodialysis were enrolled from 5 January 2014 through 22 January 2015 in the DIET-HD study and followed through 27 June 2017. Of these, 8110 (83%) were included in the analysis. Patients with an erroneous or missing identification code to allow data linkage [n = 1224 (13%)] and those with insufficient or implausible dietary responses [n = 423 (4%)] were excluded (**Figure 1**).

Figure 1: Flow chart of participation



Baseline characteristics

The mean age of the cohort was 63.1 years (standard deviation 15.0 years). Overall, 4691 (58%) were men, 2068 (33%) were former or current smokers, 934 (15%) engaged in daily physical activity, 2332 (32%) had diabetes, 838 (12%) had experienced myocardial infarction and 634 (9%) had experienced stroke. Participants had been treated with hemodialysis for a median period of 3.6 years (interquartile range 1.7 to 6.8) (**Table 1**).

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The median intake of n-3 PUFA was 1.2 (0.3 to 2.4) grams per week. Overall, 31% of participants did not consume any fish on a weekly basis, 46% consumed ≤1 serving, and 23% ≥ 2 servings each week. There was substantial variability in the dietary intake of n-3 PUFA across countries (**Table 2**). The median weekly intake of n-3 PUFA ranged from 0.2 (0.1 to 0.6) grams in Argentina and 3.1 (2.1 to 4.3) grams in Sweden. The median country-level n-3 PUFA intake was lower than the minimum recommended value for cardiovascular prevention (1.75 grams per week) in nine out of eleven countries.

Table 1: Baseline characteristics of participants

Variable	**Overall (N=8110)**
Demographics	
Age (years) (n=8110)	63.1 (15.0)
Male (n=8110)	4691(57.8)
Country (n=8110)	
Portugal	1777 (21.9)
Argentina	1204 (14.9)
Turkey	1107 (13.7)
Spain	1041 (12.8)
Romania	1000 (12.3)
Hungary	554 (6.8)
Italy	543 (6.7)
Poland	434 (5.4)
France	221 (2.7)
Germany	178 (2.2)
Sweden	51 (0.6)
Socio-economic characteristics	

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Variable	Overall (N=8110)
Married/life partner (n=6095)	4127 (67.7)
Secondary education (n=6090)	2699 (44.3)
Daily physical activity (n=6199)	934 (15.1)
Wait-listed for transplant (n=8094)	1496 (18.5)
Current or former smoker (n=6280)	2068 (32.9)
Clinical characteristics	
Body-Mass Index (kg/m^2) (n=7872)	
Underweight (<18.5)	365 (4.6)
Normal range (18.5-24.9)	3309 (42.0)
Pre-obese (25.0-29.9)	2659 (33.8)
Obese (≥30.0)	1539 (19.6)
Hypertension (n=7317)	6219 (85.0)
Diabetes (n=7280)	2332 (32.0)
Congestive heart failure (n=7272)	1388 (19.1)
Myocardial infarction (n=7236)	838 (11.6)
Stroke (n=7230)	634 (8.8)
Pulmonary disease (n=8108)	940 (11.6)
Depression (n=7218)	757 (10.5)
Gastrointestinal disease (n=8108)	1763 (21.7)
Charlson comorbidity score (n=8108)	6 (4-8)
Laboratory variables	
Albumin, g/L (n=6167)	39.8 (3.8)
Phosphorus, mg/dL (n=7869)	4.7 (1.4)
Calcium, mg/dL (n=7870)	8.9 (0.7)
Hemoglobin, g/dL (n=7869)	11.1 (1.3)

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Variable	Overall (N=8110)
Dialysis characteristics	
Arterio-venous fistula (n=8051)	6481 (80.5)
Time on dialysis (years) (n=8108)	3.6 (1.7-6.8)
Kt/V urea (n=7818)	1.7 (0.3)

Continuous data are expressed as mean (standard deviation), median (25th, 75th quartile) or number (percentage). Body Mass Index categories are defined according to the World Health Organization. Anti-hypertensive includes angiotensin converting enzyme or angiotensin II receptor blocker.

Table 2. Country-specific dietary n-3 PUFA intake and incidence (per 100,000 person-days) of cardiovascular and all-cause mortality

Country	Cardiovascular mortality	All-cause mortality	n-3 PUFA Median, grams per week (IQR)
Argentina	16	38	0.2 (0.1-0.6)
France	9	32	1.5 (0.5-2.5)
Germany	4	29	1.4 (0.3-2.2)
Hungary	25	46	0.4 (0.2-1.3)
Italy	8	27	1.4 (0.3-2.7)
Poland	15	35	1.3 (0.3-2.4)
Portugal	7	26	2.4 (1.2-6.9)
Romania	18	32	1.3 (0.2-1.8)
Spain	7	32	1.5 (0.5-3.4)
Sweden	5	16	3.1 (2.1-4.3)
Turkey	11	22	1.2 (0.3-1.5)

n-3 PUFA: long chain n-3 polyunsaturated fatty acids (calculated as sum of eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid). IQR: interquartile range.

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Cardiovascular and all-cause mortality

During a median follow up of 2.7 years (18,666 person-years), 2087 deaths (26%) were recorded, of which 829 (40%) were attributable to cardiovascular causes. The incidence of cardiovascular and all-cause mortality varied considerably by country and was highest among patients in Eastern European countries (Hungary, Poland, Romania, Turkey) and Argentina and lower among patients in Northern, Central and Western European countries (Sweden, Germany, Portugal, Spain, France, Italy) (**Table 2**). In general, patients in countries with lower incidence of cardiovascular and all-cause mortality, such as Sweden (5 and 16 deaths per 100,000 person-days, respectively) and Portugal (7 and 26 deaths per 100,000 person-days) reported a higher median intake of n-3 PUFA (3.1 [interquartile range 2.1-4.3] and 2.4 [1.2-6.9] g per week, respectively), while patients in countries with higher incidence of cardiovascular and all-cause mortality, including Hungary (25 and 46 deaths per 100,000 person-days) and Argentina (16 and 38 deaths per 100,000 person-days) reported lower intake of n-3 PUFA (0.4 [0.2-1.3] and 0.2 [0.1-0.6] grams per week respectively) (**Figure 2**).

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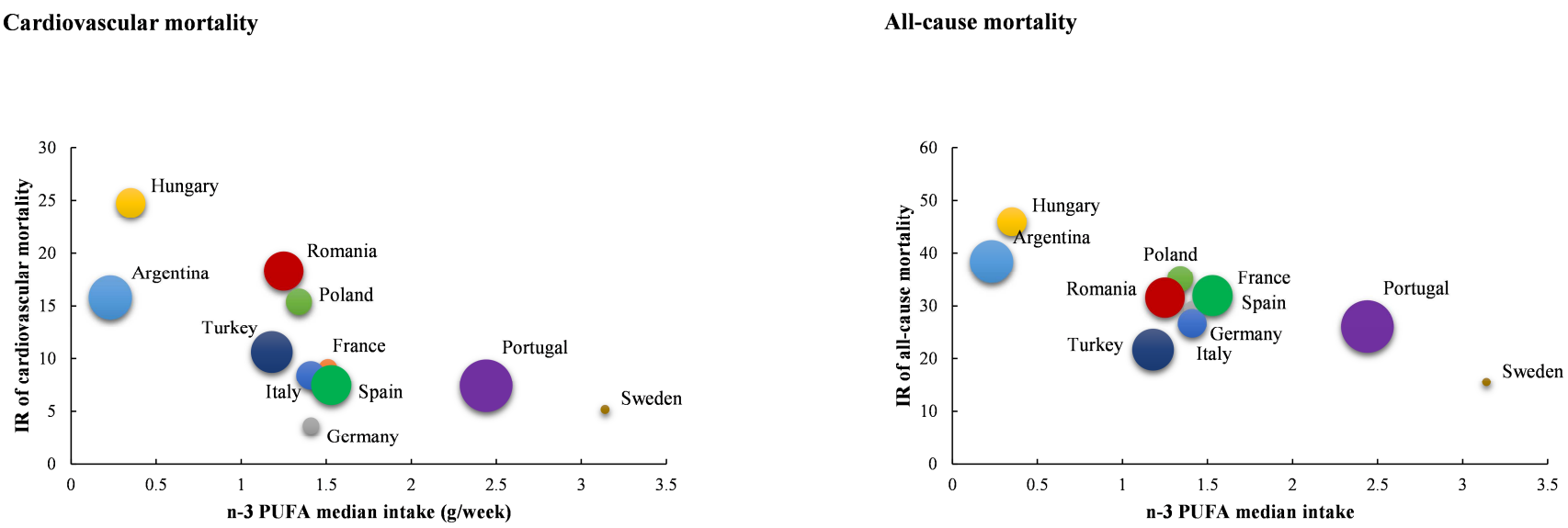
Figure 2: Unadjusted incidence (per 100,000 person-days) of cardiovascular and all-cause mortality plotted against dietary n-3 PUFA intake by country



IR: incidence rate per 100,000 person-days; n-3 PUFA: long chain n-3 polyunsaturated fatty acids (calculated as sum of eicosapentaenoic, docosapentaenoic and docosahexaenoic acids). The area of the circles is proportional to the number of participants in each country.

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Association between dietary n-3 PUFA intake and cardiovascular mortality

There was no association between dietary intake of n-3 PUFA and cardiovascular mortality. The adjusted hazard ratio (95% CI) for cardiovascular death among patients in the highest (≥1.8 grams per week) and middle tertile (0.37 to <1.8 grams per week) of dietary n-3 PUFA intake was 0.99 (0.83 to 1.19) and 0.84 (0.71 to 1.00), respectively, compared with patients in the lowest tertile (<0.37 grams per week). Similar findings were observed when considering the competing risk of other causes of death and kidney transplantation on cardiovascular mortality (**Table 3**). In subgroup analysis including only patients (N=725) achieving levels of n-3 PUFA recommended for secondary prevention (≥ 7 grams per week), no significant association between n-3 PUFA and cardiovascular mortality was observed [adjusted hazard ratio among patients in the highest (≥8.9 grams per week) and middle tertile (7.5 to <8.9 grams per week) was 1.26 (0.58-2.72) and (1.16 (0.56-2.4) respectively, compared with patients in the lowest tertile (<7.5 grams per week)]. There was no association between dietary n-3 PUFA intake and cardiovascular mortality within individual countries (**Figure 3**). Risk factors for cardiovascular mortality included older age, lower education, smoking, presence of comorbidities and higher level of phosphorus and calcium. Arterio-venous fistula vascular access, dietary fiber, serum albumin, BMI, hemoglobin and Kt/V were inversely associated with cardiovascular mortality (**Table S1**).

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Table 3: Mortality hazard ratios (95% confidence interval) by tertiles of weekly grams n-3 PUFA intake

	n-3 PUFA, grams per week		
Model	≤ 0.37	0.37 to <1.8	≥1.8
Cardiovascular mortality			
Univariate random effect	1.00	0.84 (0.71-1.00)	0.99 (0.83-1.19)
*Multivariate random effect	1.00	0.82 (0.69-0.98)	1.03 (0.84-1.26)
*Multivariate competing risk	1.00	0.82 (0.68-0.97)	1.05 (0.85-1.29)
All-cause mortality			
Univariate random effect	1.00	0.99 (0.89-1.10)	1.00 (0.89-1.12)
≠Multivariate random effect	1.00	0.96 (0.86-1.08)	1.00 (0.88-1.13)
≠Multivariate competing risk	1.00	0.97 (0.87-1.09)	1.01 (0.89-1.15)

*Adjusted for gender, education (secondary versus none/primary), smoke (former or current versus never), diabetes, myocardial infarction, vascular access type (arterio-venous fistula versus graft/catheter), Body Mass Index (categories according to WHO), albumin (tertiles), Charlson comorbidity score (quartiles), age (standard deviation increase), phosphorus, calcium, haemoglobin, KTV, fiber daily intake (tertiles), energy intake (1000 kcal per day increase). ≠As above but excluding fiber daily intake and plus time on dialysis and being wait-listed for transplant

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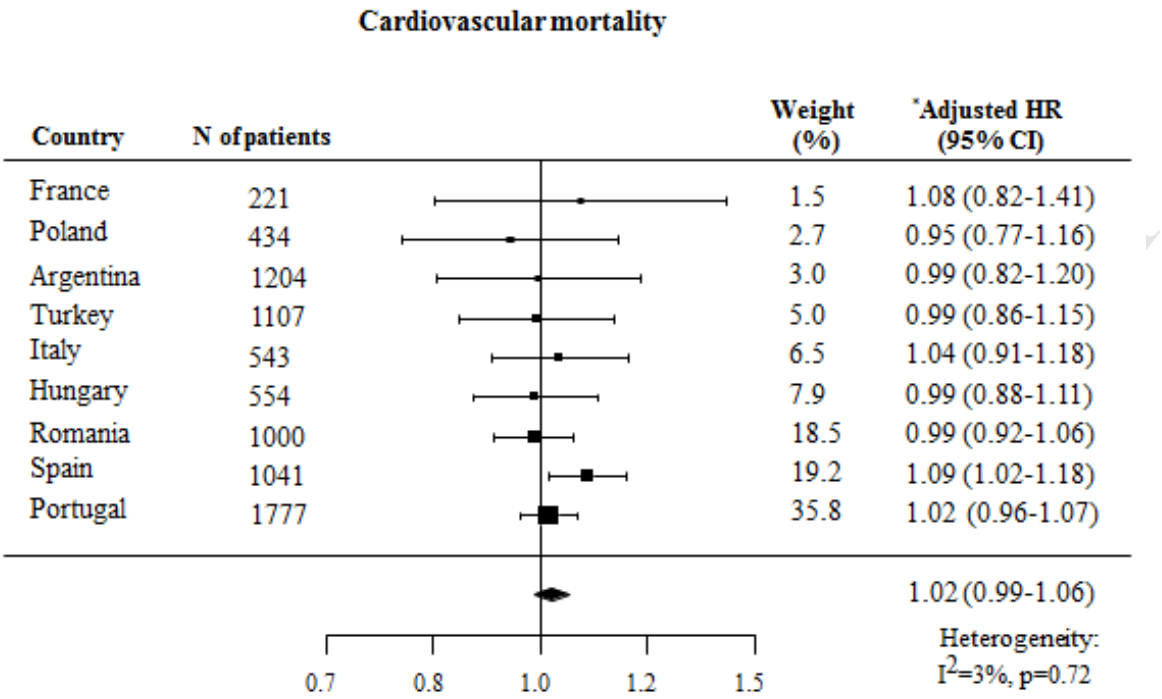
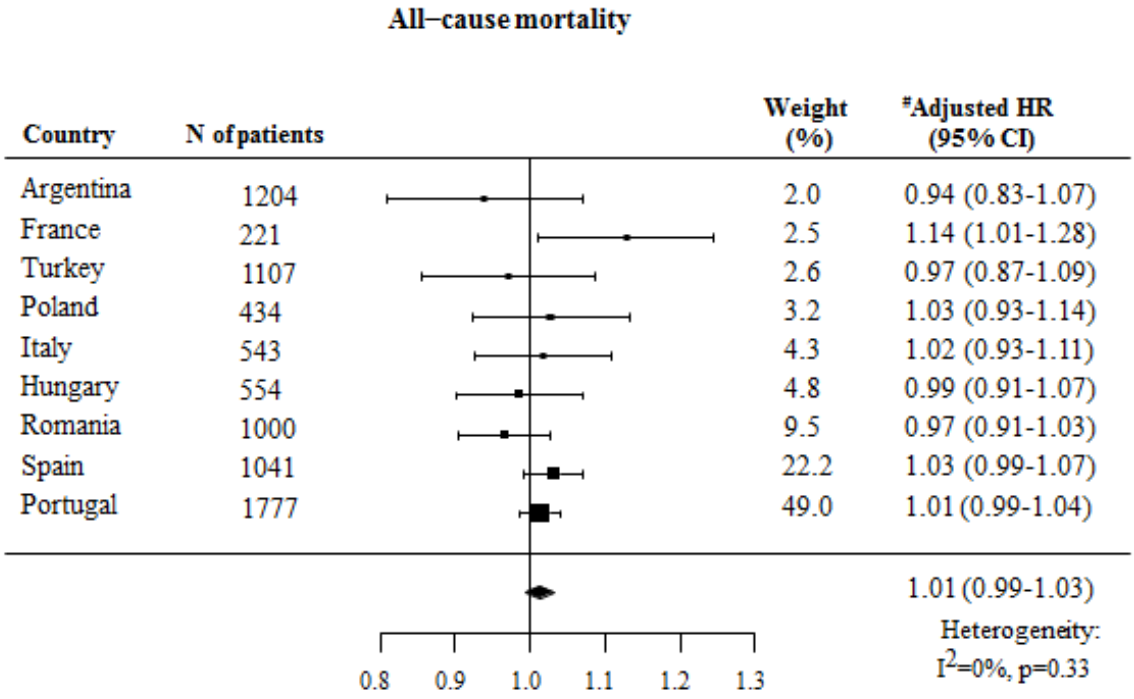
Figure 3 Association between dietary n-3 PUFA intake and cardiovascular and all-cause mortality by country





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[*]Hazard ratio adjusted for gender, education (secondary versus none/primary), smoke (former or current versus never), diabetes, myocardial infarction, vascular access type (arterio-venous fistula versus graft/catheter), Body Mass Index (categories according to WHO), albumin (tertiles), Charlson comorbidity score (quartiles), age (standard deviation increase), phosphorus, calcium, haemoglobin, KTV, fiber daily intake (tertiles), energy intake (1000 kcal per day increase). [#]As above except for fiber daily intake and plus wait-listed for transplant and time on dialysis. CI: confidence interval.

Hazard ratios for Germany and Sweden were not estimable due to the low number of outcome events.

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Association between dietary n-3 PUFA intake and all-cause mortality

Compared with patients in the lowest dietary intake of n-3 PUFA, the adjusted hazard ratio (95% CI) for all-cause mortality among those in the highest and middle n-3 PUFA tertile was 1.00 (0.88 to 1.13) and 0.96 (0.86 to 1.08), respectively. Similar findings were observed when considering the competing risk of kidney transplantation on all-cause mortality (**Table 3**). There was no association between dietary intake of either n-3 PUFA and all-cause mortality within countries (**Figure 3**). Risk factors for all-cause mortality were older age, being male, lower education, smoking, presence of comorbidities, longer time on dialysis and serum levels of phosphorus and calcium. Arterio-venous fistula vascular access, being wait-listed for renal transplantation, and higher levels of albumin, BMI, hemoglobin and Kt/V were associated with lower risks of all-cause mortality (**Table S1**).

Complete-case analysis

Similar findings were observed in complete-case analysis. 3981 participants had complete data for dietary n-3 PUFA, as ascertained by the FFQ, and for covariates and clinical outcomes, as collected within the administrative database. In this subsample of patients, the adjusted hazard ratio (95% CI) for cardiovascular death among patients in the highest and middle tertiles of n-3 PUFA intake was 1.12 (0.82 to 1.53) and 0.89 (0.66 to 1.18), respectively, compared with those in the lowest tertile. The adjusted hazard ratios (95% CI) for all-cause mortality among patients in the highest and middle tertiles of n-3 PUFA intake were 1.04 (0.87 to 1.25) and 1.02 (0.86 to 1.20), respectively, compared with those in the lowest tertile.

Discussion

In this large, multinational cohort study of dietary n-3 PUFA intake, dietary intake was generally below recommended levels for primary and secondary prevention of cardiovascular mortality. No association between the dietary n-3 PUFA intake with cardiovascular or all-cause mortality was observed among adults treated with hemodialysis. When considering an ecological approach, there was considerable variation in the dietary intake of n-3 PUFA among participating countries with an apparent inverse relationship between country-level median dietary intake of n-3 PUFA and mortality incidence. However, such ecological inferences between n-3 PUFA dietary intake and mortality were not present at the individual patient level, when controlling for clinical characteristics and energy intake.

The absence of an observed relationship between dietary n-3 PUFA intake and mortality has a number of possible interpretations, including insufficient power, duration of follow up, dietary n-3 PUFA intake below levels considered sufficient for mortality prevention, or the absence of a true relationship between n-3 PUFA dietary intake and mortality in the dialysis setting. Chief among these putative explanations may be the insufficient level of n-3 PUFA, particularly in hemodialysis patients whose mortality risk is similar in magnitude to those requiring secondary prevention and whose dietary n-3 PUFA intake in this cohort was lower than recommended for primary cardiovascular prevention. Previous interventions studies in the general population have suggested a

threshold of at least 1.75 g per week of n-3 PUFA (achieved by at least two servings of fish per week) is needed to achieve any cardiovascular benefits (38) and higher doses n-3 PUFA supplementations (≥ 7 g per week) are required for secondary cardiovascular prevention strategy (20, 21). Only one third of the present study population consumed recommended levels of n-3 PUFA as to achieve primary cardiovascular prevention, and less than 10% of study participants achieved the level of n-3 PUFA recommended for secondary prevention. The risk of cardiovascular disease within the hemodialysis population is very high, and might therefore require higher dose n-3 PUFA, analogous to secondary prevention, and which could only be achieved through supplementation. Although no significant association between n-3 PUFA and cardiovascular mortality was observed in our subgroup analysis including patients with secondary prevention levels of n-3 PUFA, the number of observations within this subgroup was too small to exclude a beneficial effect of higher dose n-3 PUFA. Existing randomized controlled trials of higher dose n-3 PUFA supplementation in patients on hemodialysis have reported no significant effect on all-cause mortality and arteriovenous fistula failure, but reduced risk of thrombosis in arteriovenous grafts and cardiovascular events (39-42). None have assessed the impact of n-3 PUFA supplementation on cardiovascular mortality as the primary outcome in this population (26, 27).

The finding of low dietary intake of n-3 PUFA in our cohort is consistent with previous studies reporting a lower intake of n-3 PUFA in the hemodialysis setting compared to the dietary guidelines for cardiovascular risk reduction (43, 44). Low dietary n-3 PUFA intake is also consistent with lower energy and

other nutrient intake, including protein and fiber, by hemodialysis patients as compared to recommendations for cardiovascular prevention in the general population, and renal nutrition recommendations (45-47). Impaired appetite caused by uremia and comorbid illness (48), and financial constraints (49) experienced by hemodialysis patients can contribute to these deficiencies in nutritional intake.

The present study has several strengths. It is the primary analysis of the DIET-HD study, a large multinational prospective cohort study investigating putative determinants of adverse clinical outcomes for patients on hemodialysis. In particular, the DIET-HD study examines the role of diet in the hemodialysis setting that is an important area of research uncertainty prioritized by healthcare professionals and patients (50). The population was geographically diverse thereby enhancing the generalizability of the findings. To date, no data are available on dietary intake of n-3 PUFA and its association with mortality in hemodialysis patients across different countries using a common FFQ. This was the first FFQ specifically designed to allow international comparisons and that included local foods for each participant country.

Limitations of the study should be also considered. First, ascertainment errors were possible due to the use of a self-reported questionnaire. Second, despite the high response rate to the FFQ, the exclusion of patients with erroneous or missing identification code (13%) raised the potential for selection bias. Third, information regarding the potential consumption of PUFA supplementation was not available. In addition, the single baseline measurement of dietary n-3 PUFA intake may be not reflective of the actual cumulative intake over time both

before the study commended and during follow up. Moreover, blood levels of n-3 PUFA were not measured and may reflect more accurately longer term consumption and myocardial n-3 PUFA composition. However, the correlation between dietary intake of n-3 PUFA ascertained by the GA^{2}LEN FFQ and corresponding levels in plasma has been validated in a previous pilot study (37) reporting results in agreement with those of other validation studies of dietary n-3 PUFA intake from FFQs with plasma levels (51, 52).

In summary, the dietary intake of n-3 PUFA in adults treated with hemodialysis is below the recommended levels for primary and secondary cardiovascular prevention. At these levels of n-3 PUFA intake, there was no demonstrable association between dietary n-3 PUFA and mortality which may indicate either that n-3 PUFA does not improve cardiovascular outcomes in hemodialysis patients or that supplementation is required to improve clinical outcomes.

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Authors' Contributions

Conception and design: VMS, GW, MR, SCP, KC, VGL, JCC, and GFMS; Data acquisition: MR, PN, LG, AMM, MG, RG, EC, TE, AGB, DDC, DT, MT, ABS, JD, PS, SH, MH, EF, CW; Data analysis: VMS, GW, ATP, GFMS; Data interpretation: all authors; Study supervision and mentorship: GFMS, JCC, GW, SCP. Each author contributed important intellectual content during manuscript drafting or revision and gave final approval of the version to be submitted.

Conflict of interest

Authors have nothing to declare.

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Figures legend

Figure 1: Flow chart of participation

Figure 2: Unadjusted incidence (per 100,000 person-days) of cardiovascular and all-cause mortality plotted against dietary n-3 PUFA intake by country

Figure 3 Association between dietary n-3 PUFA intake and cardiovascular and all-cause

Tables legend

Table 1: Baseline characteristics of participants

Table 2. Country-specific n-3 PUFA dietary intake and incidence (per 100,000 person-days) of cardiovascular and all-cause mortality

Table 3: Mortality hazard ratios (95% confidence interval) by tertiles of weekly grams n-3 PUFA intake

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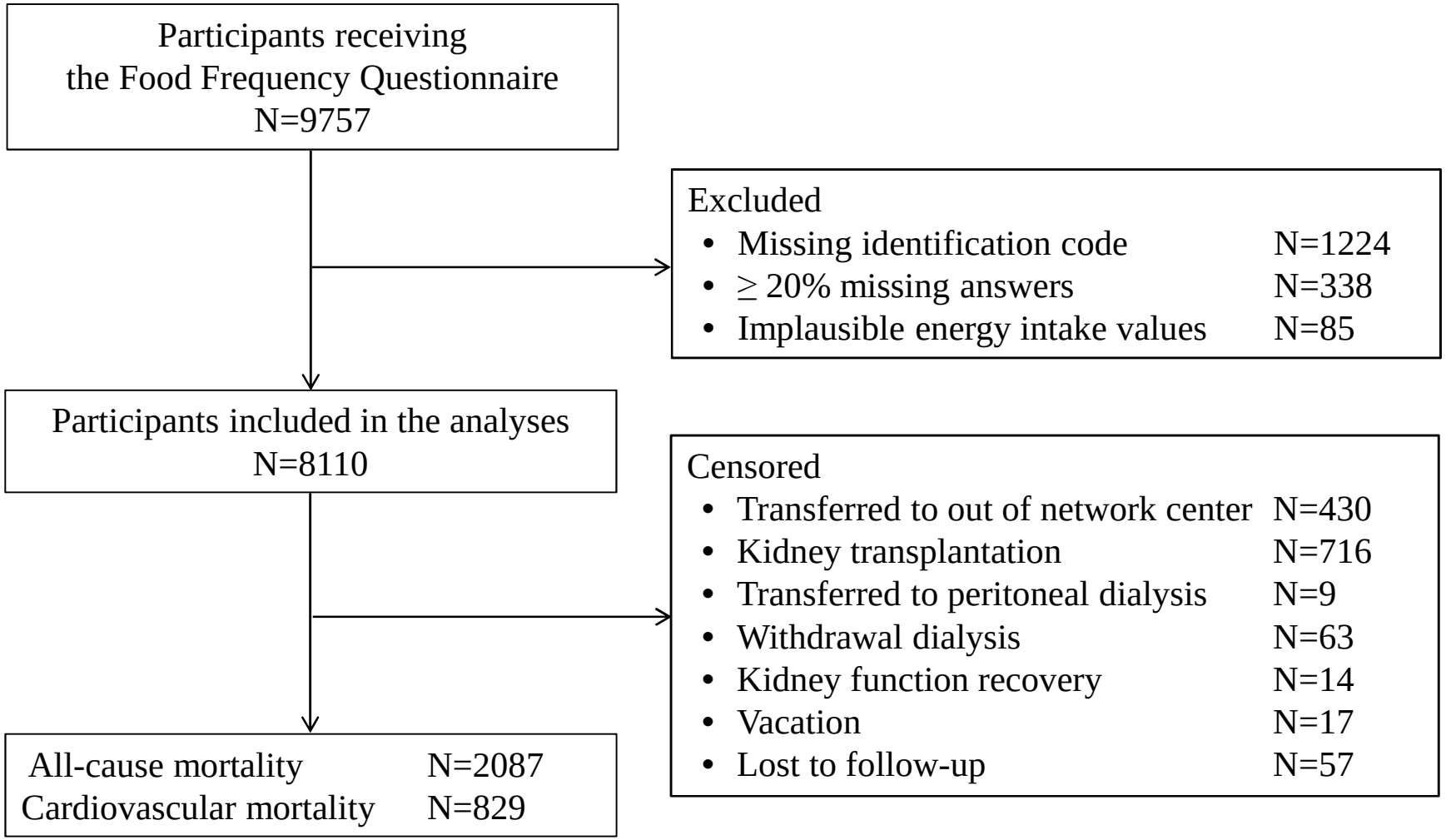

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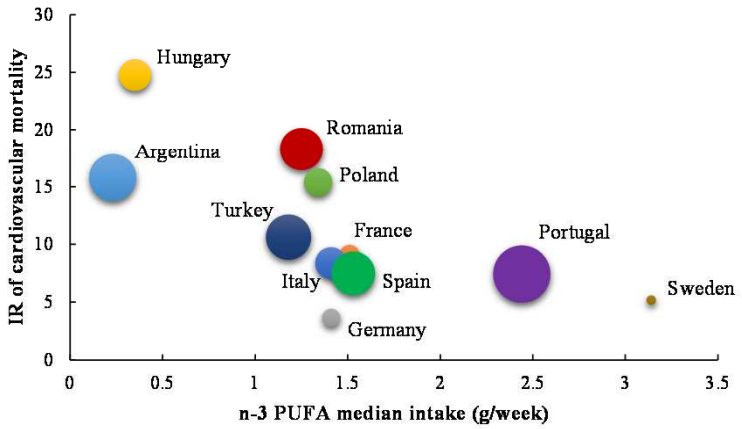

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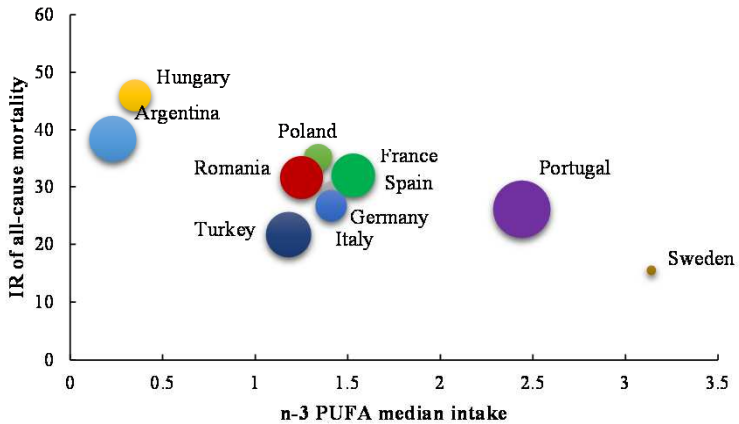

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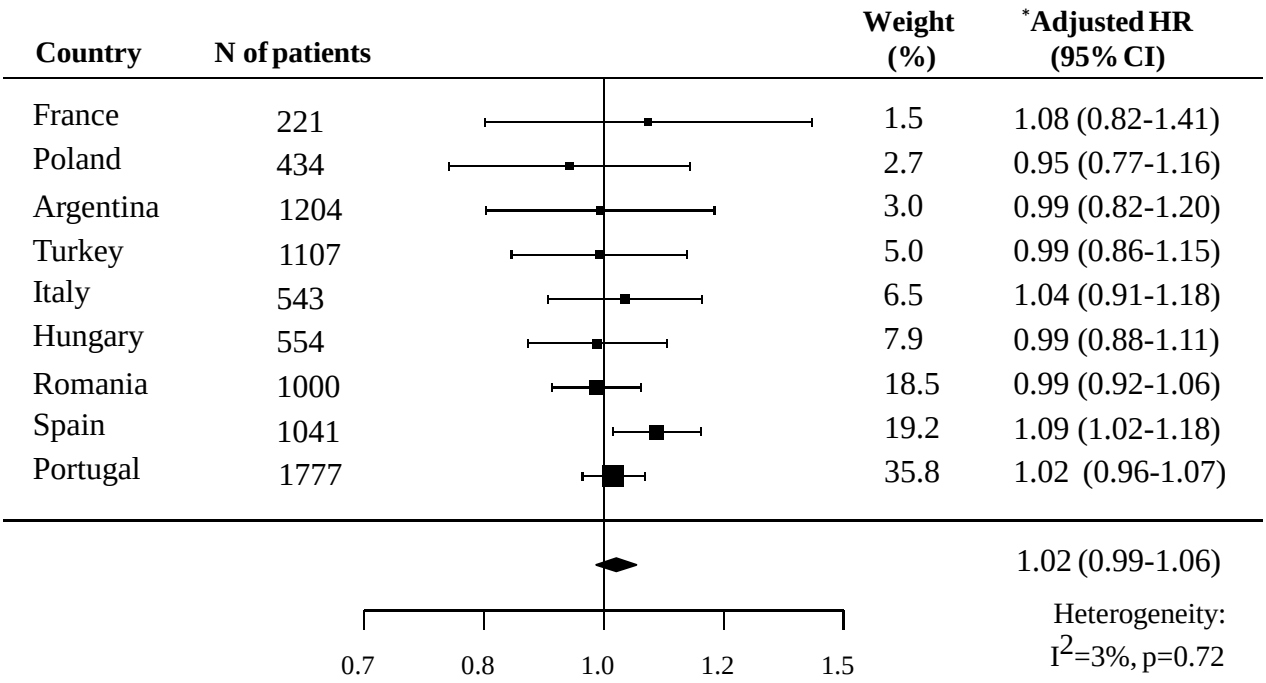

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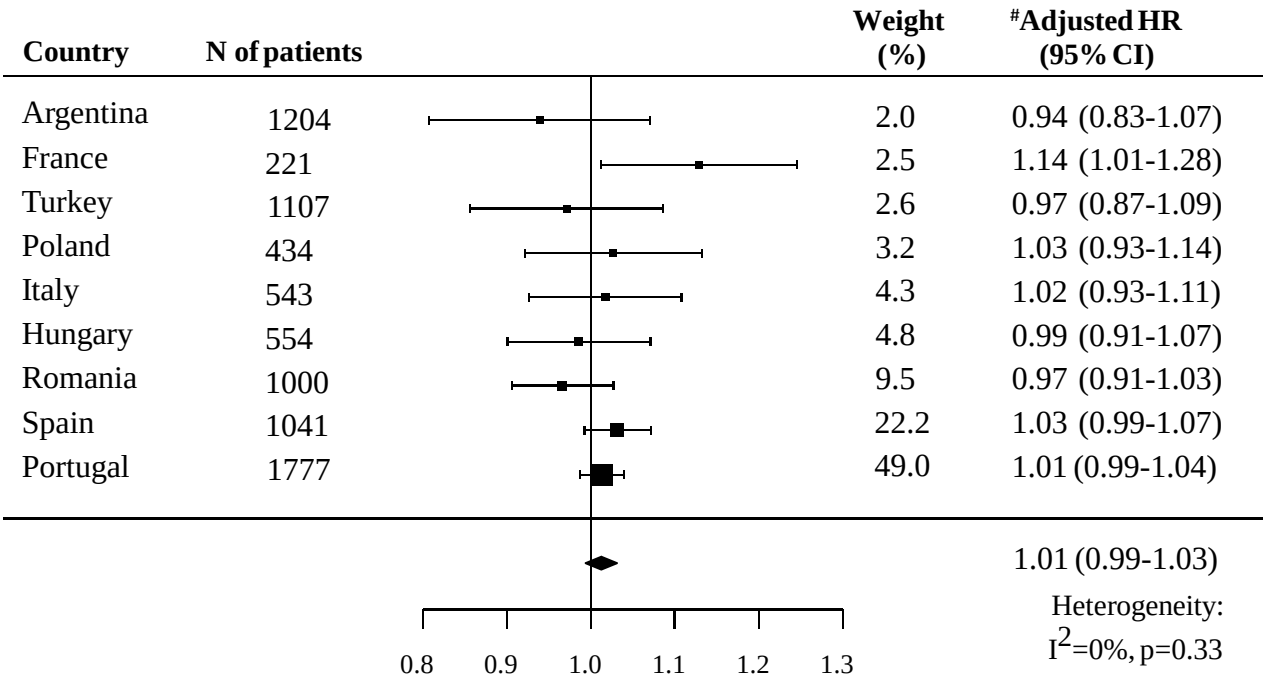

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