# Plasma n-6 Polyunsaturated Fatty Acid Levels and Survival in Renal Transplantation

Skaare, Helga; Svensson, My; Jenssen, Trond; Åsberg, Anders; Schmidt, Erik Berg; Chandra, Anupam; Ueland, Thor; Mollnes, Tom Eirik; Hartmann, Anders; Eide, Ivar Anders

*Published in:*
Journal of Renal Nutrition

*DOI (link to publication from Publisher):*
10.1053/j.jrn.2018.02.008



*Publication date:*
2018

*Document Version*
Accepted author manuscript, peer reviewed version

# PLASMA n-6 POLYUNSATURATED FATTY ACID LEVEL AND SURVIVAL IN RENAL TRANSPLANTATION

*Helga Skaare.[1] My Svensson.[1,2] Trond Jenssen.[3,4] Anders Åsberg.[3,5,6] Erik B. Schmidt.[7] Anupam Chandra.[1] Thor Ueland.[2,8,9] Annika Michelsen.[8] Tom Eirik Mollnes.[9,10,11,12] Anders Hartmann.[2,3] Ivar Anders Eide.[1,3]*

[1] Department of Renal Medicine, Akershus University Hospital, Lørenskog, Norway.
[2] Institute of Clinical Medicine, Faculty of Medicine, The University of Oslo, Oslo, Norway.
[3] Department of Transplantation Medicine, Section of Nephrology, Oslo University Hospital, Rikshospitalet, Oslo, Norway.
[4] Metabolic and Renal Research Group, UiT The Arctic University of Norway, Tromsø, Norway.
[5] School of Pharmacy, University of Oslo, Oslo, Norway.
[6] The Norwegian Renal Registry, Oslo University Hospital, Rikshospitalet, Oslo, Norway.
[7] Department of Cardiology, Aalborg University Hospital, Aalborg, Denmark.
[8] The Research Institute of Internal Medicine, Oslo University Hospital, Rikshospitalet, Oslo, Norway.
[9] KG Jebsen Thrombosis Research and Expertise Centre, UiT The Arctic University of Norway, Tromsø, Norway.
[10] Institute of Immunology, Faculty of Medicine, The University of Oslo, Oslo, Norway.
[11] Department of Laboratory Medicine, Nordland Hospital, Bodø, Norway.
[12] Centre of Molecular Inflammation Research, Norwegian University of Science and Technology, Trondheim, Norway.

**Corresponding author:**
Helga Skaare, Department of Renal Medicine, Akershus University Hospital, Lørenskog, Norway.
Helga.skaare@ahus.no, +47 67966027

**Funding**
This research project was funded by participating hospitals and did not receive any additional funding from public, commercial or not-for-profit funding agencies.

**Disclosures**
The authors declare no disclosures.

**Author Contributions:**
H.S, M.S and I.A.E designed the study.
T.J, A.Å, A.H and I.A.E collected data from patient records for patients transplanted at Oslo University Hospital Rikshospitalet between 30th of September 1999 and 13th of October 2011.
E.B.S was responsible for fatty acid analyses.
T.U, T.E.M and A.M performed the inflammatory marker analyses.
I.A.E and A.C analysed the data.
H.S, M.S, A.C and I.A.E edited and T.J, A.Å, A.H, E.B.S, T.U, T.E.M and A.M co-edited the manuscript.
H.S, M.S, A.C, I.A.E, T.J, A.Å, A.H, E.B.S, T.U, T.E.M and A.M approved the final version of the manuscript.
H.S submitted the manuscript.

**Abbreviations:**

| | |
|---|---|
| AA | Arachidonic acid |
| CV | Cardiovascular |
| DM | Diabetes mellitus |
| FA | Fatty acid |
| fPG | Fasting plasma glucose |
| GDF-15 | Growth differential factor 15 |
| HDL | High-density lipoprotein |
| IL | Interleukin |
| LA | Linoleic acid |

| | |
|---|---|
| LDL | Low-density lipoprotein |
| PUFA | Polyunsaturated fatty acid |
| RTR | Renal transplant recipient |
| T2DM | Type 2 diabetes mellitus |
| wt% | Weight percentage |
| 2hPG | 2 hours post-challenge plasma glucose |

**Acknowledgements**
We acknowledge the skilled assistance of Sebastian Müller and Helga Grimstad Sørhøy at the Laboratory of Renal Physiology, Oslo University Hospital, Rikshospitalet. We thank Dr. Stein Bergan at Oslo University Hospital, Rikshospitalet for laboratory analyses and Dr. Torbjørn Leivestad at The Norwegian Renal Registry, Oslo University Hospital, Rikshospitalet for provision of register data. We thank Rikke B. Eschen, Birthe H. Thomsen and Anne-Mette Christensen at The Lipid Research Laboratory, Aalborg University Hospital, Denmark for performing the fatty acid analyses.

**Key words**: n-6 PUFA, linoleic acid, arachidonic acid, survival, inflammation, glucose, transplantation.

**Practical applications of the present study**
Plasma levels of linoleic acid were not related to patient or graft survival after renal transplantation. Our findings suggest a beneficial effect of linoleic acid consumption on inflammation and glucose metabolism.

## Abstract

**Objective**: The major n-6 polyunsaturated fatty acids linoleic acid (LA) and arachidonic acid (AA) play a role in inflammation and glucose metabolism, which could influence patient and renal transplant survival.

**Design**: Single center cohort study.

**Setting and study subjects**: Cohort of 1988 Norwegian renal transplant recipients.

**Methods and main outcome measures**: We assessed associations between plasma levels of LA and AA at baseline, measured by gas chromatography, and patient and graft survival, as well as inflammation and cardiovascular risk markers.

**Results**: During follow-up (median of 9.6 years), 595 patients died and 805 renal transplants were lost, either due to recipient death or graft failure. In multivariable survival analysis, we found no associations with mortality for plasma levels of LA (HR 0.99, 95% CI 0.96-1.01) or AA (HR 1.01, 95% CI 0.96-1.06). No associations were found for either cardiovascular mortality, overall graft loss or death censored graft loss. Levels of plasma glucose, pro-glycemic marker chemerin and pro-inflammatory marker growth differentiation factor 15 were inversely associated with plasma LA level and positively associated with plasma AA levels in multivariable analysis.

**Conclusions**: We found no associations between plasma levels of LA or AA and patient or graft survival. Plasma levels of LA and pro-glycemic indices were inversely associated, signaling a possible beneficial effect of LA consumption for prevention of type 2 diabetes mellitus in renal transplant recipients.

## Introduction

Linoleic acid (LA) and arachidonic acid (AA) are the major n-6 polyunsaturated fatty acids (PUFAs) in the diet.[1] While LA is an essential fatty acid (FA) and thus entirely dependent on dietary intake, AA can in addition be obtained by endogenous metabolism of LA. LA is mainly found in nuts and vegetable oils and AA in eggs, poultry and meat.[2 3] Reports from other patient populations suggest that LA may prevent development of type 2 diabetes mellitus (T2DM) [4] possibly through beneficial effects on insulin sensitivity.[5] The mechanism is not completely revealed but the anti-inflammatory properties of LA is likely involved. In contrast, AA and its metabolites also have pro-inflammatory and pro-glycemic effects[6], and both hyperglycemia and inflammation are independent risk factors for cardiovascular (CV) disease, which is the leading cause of death in renal transplantation recipients (RTRs).[7]

To our knowledge, no data exist on the long-term impact of n-6 PUFA consumption in patients undergoing organ transplantation and no previous study has assessed associations between plasma n-6 PUFA levels and inflammatory or CV risk markers in a renal transplant cohort.

The aim of the present study was to assess whether plasma levels of LA and AA were associated with mortality and graft loss in RTRs. In addition, we assessed cross-sectional associations between plasma LA and AA levels and various inflammatory and CV risk markers.

## Materials and methods

### Study population and clinical data

The study population and design have previously been described in detail.[8-10] In short, 1988 out of 2345 eligible renal transplant recipients, transplanted at Oslo University Hospital, Rikshospitalet, during September 1999 through October 2011, were included in the study. Patients not eligible for participation in the study included patients under the age of 16 years, patients transferred to their local hospitals within 10 weeks after transplantation, and patients who suffered graft failure or died within the first 10 weeks following renal transplantation. The immunosuppressive protocol was based on a combination of a calcineurin inhibitor, prednisolone and a cell proliferation inhibitor, with some variation during the study period. Until 2007, the calcineurin inhibitor of choice was cyclosporine, followed by a period where the choice of calcineurin inhibitor (tacrolimus or cyclosporine) was based on recipient age and CV risk profile. Tacrolimus has been the calcineurin inhibitor of choice from 2012, but patients started on cyclosporine were not switched to tacrolimus. Statin became standard therapy during the study period, with a coverage from 30% in 2000 to >70% from 2007 and onwards.

The study was approved by the Regional Committees for Medical and Health Research Ethics in Norway and was performed in accordance with the Declaration of Helsinki.

### Data collection and laboratory methods

Clinical data were obtained from medical records. The Norwegian Renal Registry, which includes all Norwegian patients on renal replacement therapy, provided endpoint data. Overall renal graft loss included both functional grafts lost due to recipient death and death censored graft loss (return to dialysis therapy or renal re-transplantation). At 10 weeks post-transplant, all patients underwent a uniform clinical investigation at the Laboratory for renal physiology at Oslo University Hospital, Rikshospitalet. Blood was sampled and aliquots of biobanked samples were sent to The Lipid Research Center, Aalborg University Hospital for FA analysis by gas chromatography as previously described.[8] Individual FAs were identified and quantified as weight percentage (wt%) of total plasma FA. Plasma inflammation markers were analyzed at The Research Institute of Internal Medicine at Oslo University Hospital, Rikshospitalet and at The Research Laboratory, Nordland Hospital, Bodø using enzyme immunoassay and multiplex cytokines assay as appropriate.[9] In non-diabetic patients, fasting plasma glucose (fPG) was measured and 2-hours post-challenge plasma glucose (2hPG) was obtained during a standard OGTT. Inflammatory markers were measured in patients transplanted from 2007. Other parameters were measured from 1999.

### Statistics

The main statistical approach was survival analysis, using Cox proportional hazard regression with all-cause mortality and overall graft loss as outcomes, and proportional hazard regression model for the subdistribution of competing risks as described by Fine and Grey [11] for outcomes CV mortality and death censored graft loss. We estimated crude, age and gender adjusted and multivariable adjusted hazard ratios (HR) of reaching mortality and graft loss end-points. Proportional hazard assumptions were checked by inspection of the log-log survival time plots and by a formal hypothesis test (Schoenfeld residuals). Cross-sectional associations between plasma phospholipid levels of LA and AA and inflammatory biomarkers and CV risk markers at 10 weeks post-transplant were assessed by age and gender adjusted and multivariable adjusted linear regression. Candidate variables were included in final regression models in a stepwise forward manner (inclusion criteria of $p<0.10$). Plasma levels of either LA or AA were forced into the final model. Statins were paused during the first 3 months after renal transplantation and thus do not influence cross-sectional associations. Only patients without overt DM underwent measurements of fPG and 2hPG. Associations between plasma phospholipid LA and AA levels and various inflammatory and CV risk markers are presented as unstandardized regression coefficient (Unstd. β-coeff.), corresponding 95% confidence intervals (CI) and standardized regression coefficient (Std. β-coeff.). Due to non-normal distribution, dialysis vintage and inflammatory biomarkers were logarithmically transformed before they were entered as variables in linear regression analyses. Thus, for inflammatory biomarkers, the presented Unstd. β-coeff. and corresponding 95% CI represents the anti-logarithm of obtained results. SPSS® version 24.0 (IBM, NY, US) and STATA® version 14.0 (Stata Corp, College Station, Texas, US) were used for the statistical analysis. A two-sided p-value of $< 0.05$ was considered statistically significant.

## Results

### Demographics

Levels of AA and LA in plasma were inversely correlated (Pearsons'r = -0.30). As can be seen in Table 1, multiple baseline characteristics were associated with AA and LA levels. In particular, patients with high LA and AA were younger, male, had better renal function, used different immunosuppressive agents, had a higher prevalence of DM at the time of transplantation and a different biochemical profile including lower total cholesterol. Patients with high LA had lower body mass index (BMI) while patients with high AA had higher BMI.

During follow-up (19 906 person-years with a median follow-up of 9.6 years) there were 595 deaths (30%), including 225 deaths from cardiovascular causes. A total of 805 renal grafts were lost, either due to recipient death (n=497) or death censored graft loss (n=308).

### Linoleic acid

In univariate analysis, we found significant inverse associations between plasma LA levels and overall graft loss and all-cause and CV mortality, but not death censored graft loss (Table 2). After adjustment for traditional and transplant specific risk factors, no significant associations were found between plasma LA levels and mortality or graft loss end-points (Table 2). Subgroup analyses revealed no interactions between LA levels and DM or indices of glucose metabolism on long-term outcomes.

In a multivariable linear regression analysis, plasma levels of LA were inversely associated with fPG and 2hPG and positively associated with plasma high-density lipoprotein (HDL) cholesterol levels (Table 3). Plasma LA levels were negatively associated with levels of growth differential factor 15 (GDF-15) and chemerin (Table 4).

### Arachidonic acid

Plasma levels of AA were not associated with all-cause mortality (crude HR 0.98 [0.93-1.02], multivariate HR 1.01 [0.96-1.06]) or overall graft loss (crude HR 1.00 [0.96-1.04], multivariate HR 1.01 [0.96-1.05]). Multivariable linear regression revealed that plasma AA was negatively associated with plasma HDL

cholesterol levels and positively associated with fPG and 2hPG (Table 3). Further, regression analysis showed positive associations between plasma AA level and GDF-15 and chemerin (Table 4).

## Discussion

Plasma levels of LA and AA levels were not associated with either patient or graft survival after adjustment for confounders. We found negative associations between plasma LA levels and pro-glycemic and pro-inflammatory indices, while positive associations with the same markers were found for plasma AA levels.

### *Patient and graft survival*

High levels of LA and AA were often found in young RTRs, which inevitably will infer confounding, and crude survival data should be interpreted with caution. After adjustment for multiple confounders, we found no significant associations between plasma levels of LA or AA, mortality and graft loss, suggesting no survival benefit from enriching the diet with LA, despite possible beneficial anti-glycemic and anti-inflammatory effects. Much of the concern regarding n-6 PUFA consumption is related to unfavorable metabolic and inflammatory effects of eicosanoid deriving from AA. The findings of this study do not support this concern, as no unfavorable association with long-term outcomes was found in patients with high plasma levels of AA.

### *Inflammation, lipid and glucose metabolism*

We found that higher plasma levels of LA are associated with lower fPG and 2hPG, even after adjustment for potential confounders. A similar cross-sectional correlation between plasma LA and glucose levels were found in a recent population-based study from the Netherlands.[12] In the present study, plasma LA levels showed a particularly strong negative association with chemerin, a cytokine involved in adipocyte differentiation, glucose and lipid metabolism and immune regulation.[13 14] Chemerin levels normalize early after renal transplantation and do not reflect pre-transplant levels at 10 weeks post-transplant.[15] Since both pre- and post-transplantation DM is frequent and associated with increased mortality,[16] the potential anti-glycemic properties of LA would be of particular interest in an organ transplant cohort.

In humans, LA has been shown to lower low-density lipoprotein (LDL) cholesterol, and higher PUFA levels are associated with a reduced ratio of total to HDL cholesterol.[17] Reports from cohort studies in the general population indicate that LA consumption lower the risk of CV disease.[18 19] We found a positive association between plasma LA levels and HDL cholesterol levels in this cohort, but no association with LDL cholesterol.

Higher plasma LA levels were also associated with lower GDF-15 levels, a transforming growth factor-beta superfamily stress-response cytokine, associated with DM, cancer, CV disease and mortality.[20] In humans, short-term high dose LA consumption reduced circulating pro-inflammatory cytokine levels.[21] However, we did not detect an association between plasma LA levels and markers of tumor necrosis factor pathway activation. For AA, there is firm evidence for a pro-inflammatory effect,[6] and we found a positive association with GDF-15 levels.

We have previously reported that higher plasma levels of LA were associated with improved renal graft function during the first year after transplantation.[22] However, we did not find an association between plasma LA levels and death censored graft loss in this cohort.

### *Strengths and limitations*

In addition to the observational design, there are several limitations in the present study. We lack data on dietary habits to adjust for the full matrix of nutrients. There might be residual confounding from a potentially healthier lifestyle associated with higher levels of LA. Plasma FA composition at 10 weeks post-transplant may not reflect long-term FA profile and this concern extends to plasma inflammatory biomarkers and CV risk markers, also analyzed at the same point of time. Circulating inflammatory cytokines may stem from sources outside the renal graft and we lack data on urinary inflammation markers to check for

consistency. Our findings may not apply to other patient populations with different dietary habits. This study also has substantial strengths, including a large and well-defined population, uniform clinical procedures, a long follow-up period, few missing data and adjustment for several traditional and transplant-specific mortality and graft loss risk factors.

## Conclusion

In this renal transplant cohort, we found no associations between plasma levels of LA and survival. Plasma LA levels were negatively associated with fPG, 2hPG and chemerin, while plasma AA level showed positive associations with these glycemic indices, suggesting an anti-glycemic effect of LA consumption and a pro-glycemic effect of AA consumption. Inverse associations with GDF-15 signals that LA might possess anti-inflammatory effects.

## References


1. Burr GO, Burr, M. M. A New Deficiency Disease Produced by the Rigid Exclusion of Fat from the Diet. J Biol Chem 1929;82:345-67.
2. Whelan J, Fritsche K. Linoleic acid. Adv Nutr 2013;4(3):311-2.
3. Schwingshackl L, Schwedhelm C, Hoffmann G, et al. Food groups and risk of all-cause mortality: a systematic review and meta-analysis of prospective studies. Am J Clin Nutr 2017;105(6):1462-73.
4. Forouhi NG, Imamura F. Association of Plasma Phospholipid n-3 and n-6 Polyunsaturated Fatty Acids with Type 2 Diabetes: The EPIC-InterAct Case-Cohort Study. PLoS Med 2016;13(7):e1002094.
5. Imamura F, Micha R, Wu JH, et al. Effects of Saturated Fat, Polyunsaturated Fat, Monounsaturated Fat, and Carbohydrate on Glucose-Insulin Homeostasis: A Systematic Review and Meta-analysis of Randomised Controlled Feeding Trials. PLoS Med 2016;13(7):e1002087
6. Calder PC. Dietary arachidonic acid: harmful, harmless or helpful? Br J Nutr 2007;98(3):451-3.
7. Israni AK, Snyder JJ, Skeans MA, et al. Predicting coronary heart disease after kidney transplantation: Patient Outcomes in Renal Transplantation (PORT) Study. Am J Transplant 2010;10(2):338-53.
8. Eide IA, Jenssen T, Hartmann A, et al. The association between marine n-3 polyunsaturated fatty acid levels and survival after renal transplantation. Clin J Am Soc Nephrol 2015;10(7):1246-56.
9. Eide IA, Asberg A, Svensson M, et al. Plasma Levels of Marine n-3 Fatty Acids Are Inversely Correlated With Proinflammatory Markers sTNFR1 and IL-6 in Renal Transplant Recipients. J Ren Nutr 2016; 27:161-168.
10. Eide IA, Dahle DO, Svensson M, et al. Plasma levels of marine n-3 fatty acids and cardiovascular risk markers in renal transplant recipients. Eur J Clin Nutr 2016; 70:824-830.
11. JP F. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc. 1999;94:496–509.
12. Cabout M, Alssema M, Nijpels G, et al. Circulating linoleic acid and alpha-linolenic acid and glucose metabolism: the Hoorn Study. Eur J Nutr 2016 (Epud ahead of print).
13. Bobbert T, Schwarz F, Fischer-Rosinsky A, et al. Chemerin and prediction of Diabetes mellitus type 2. Clin Endocrinol 2015;82(6):838-43.
14. Gu P, Jiang W, Lu B, et al. Chemerin is associated with inflammatory markers and metabolic syndrome phenotypes in hypertension patients. Clin Exp Hypertens 2014;36(5):326-32.
15. Rutkowski P, Sledzinski T, Zielinska H, et al. Decrease of serum chemerin concentration in patients with end stage renal disease after successful kidney transplantation. Regul Pept 2012;173(1-3):55-9.
16. Eide IA, Halden TA, Hartmann A, et al. Mortality risk in post-transplantation diabetes mellitus based on glucose and HbA1c diagnostic criteria. Transplant Int 2016;29(5):568-78.
17. Harris WS, Mozaffarian D, Rimm E, et al. Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. Circulation 2009;119(6):902-7.
18. Cho BH, Dokko RC, Chung BH. Oleic, linoleic and linolenic acids enhance receptor-mediated uptake of low density lipoproteins in Hep-G2 cells. J Nutr Biochem 2002;13(6):330-36.
19. Sato M, Yoshida S, Nagao K, et al. Superiority of dietary safflower oil over olive oil in lowering serum cholesterol and increasing hepatic mRnas for the LDL receptor and cholesterol 7alpha-hydroxylase in exogenously hypercholesterolemic (exHC) rats. Biosci Biotechnol Biochem 2000;64(6):1111-7.

20. Wollert KC, Kempf T, Wallentin L. Growth Differentiation Factor 15 as a Biomarker in Cardiovascular Disease. Clin Chem 2017;63(1):140-51.
21. Schmid A, Bala M, Leszczak S, et al. Pro-inflammatory chemokines CCL2, chemerin, IP-10 and RANTES in human serum during an oral lipid tolerance test. Cytokine 2016;80:56-63.
22. Eide IA, Dorje C, Svensson M, et al. Development of Kidney Transplant Fibrosis Is Inversely Associated With Plasma Marine Fatty Acid Level. J Ren Nutr 2017 (Epub ahead of print).

**Table 1. Baseline characteristics of study participants according to plasma levels of n-6 polyunsaturated fatty acid.**

| n-6 PUFA | | Linoleic acid | | | Arachidonic acid | | |
|---|---|---|---|---|---|---|---|
| | **All patients** | **Low levels** | **High levels** | **p** | **Low levels** | **High levels** | **p** |
| **Level (wt%)** | | < 25.00 | ≥ 25.00 | | < 7.75 | ≥ 7.75 | |
| Number of patients | 1988 | 1049 | 939 | | 995 | 993 | |
| Recipient age, *years* | 51 (14) | 54 (14) | 49(15 | <0.001 | 53 (14) | 50 (14) | <0.001 |
| Donor age, *years* | 47 (16) | 49 (16) | 45 (16) | <0.001 | 48 (16) | 47 (16) | 0.28 |
| Gender (Male), *%* | 67 | 65 | 70 | 0.01 | 65 | 70 | 0.03 |
| eGFR, *ml/min x 1.73m$^2$* | 56 (18) | 55 (19) | 58 (19) | 0.006 | 55 (18) | 59 (19) | <0.001 |
| Tacrolimus, *%* | 21 | 17 | 27 | <0.001 | 18 | 25 | <0.001 |
| Cyclosporin, *%* | 74 | 79 | 70 | <0.001 | 78 | 70 | <0.001 |
| Atherosclerotic disease, *%* | 22 | 24 | 21 | 0.25 | 20 | 25 | 0.02 |
| Diabetes mellitus, *%* | 18 | 15 | 22 | <0.001 | 15 | 21 | <0.001 |
| Current smoker, *%* | 20 | 18 | 22 | 0.04 | 21 | 19 | 0.4 |
| Number of antihypertensive drugs | | | | | | | |
| None or one, *%* | 54 | 52 | 57 | | 58 | 51 | |
| Two or three, *%* | 41 | 43 | 40 | 0.10 | 38 | 44 | 0.01 |
| Four or more, *%* | 4.4 | 4.9 | 3.8 | | 4.2 | 4.5 | |
| Body mass index, *kg/m2* | 24 (3.8) | 25 (4.0) | 24 (3.6) | <0.001 | 24 (3.7) | 25 (4.0) | <0.001 |
| Albumin, *g/dL* | 3.9 (0.4) | 4.0 (0.4) | 4.0 (0.4) | 0.09 | 3.9 (0.4) | 4.0 (0.3) | <0.001 |
| Fasting plasma glucose, *mg/dL* | 101 (36) | 99 (35) | 103 (38) | 0.02 | 101 (38) | 101 (35) | 0.94 |
| Total cholesterol, *mg/dL* | 244 (58) | 247 (60) | 240 (56) | 0.007 | 249 (56) | 239 (60) | <0.001 |
| LDL cholesterol, *mg/dL* | 158 (65) | 161 (82) | 154 (48) | 0.02 | 160 (49) | 155 (84) | 0.14 |
| HDL cholesterol, *mg/dL* | 59 (18) | 58 (18) | 60 (19) | 0.10 | 61 (19) | 57 (18) | <0.001 |
| Dialysis vintage, *months* | 9 (0-19) | 9 (0-19) | 8 (0-19) | 0.14 | 8 (0-18) | 10 (1-21) | 0.01 |
| Preemptive transplantation, *%* | 25 | 25 | 26 | 0.41 | 26 | 24 | 0.27 |
| First renal transplant, *%* | 90 | 91 | 89 | 0.10 | 90 | 90 | 0.95 |
| Living donor transplantation, *%* | 31 | 31 | 32 | 0.47 | 34 | 29 | 0.03 |
| Number of human leucocyte antigen DR mismatches | | | | | | | |
| None, *%* | 42 | 44 | 40 | | 44 | 40 | |
| One, *%* | 50 | 48 | 51 | 013 | 49 | 51 | 0.28 |
| Two, *%* | 8.4 | 7.7 | 9.2 | | 7.8 | 9.0 | |

Baseline characteristics of the study population according to levels of linoleic acid and arachidonic acid in weight percentage (*wt%*) of total plasma phospholipid fatty acids. Results are presented as proportions for categorical data, median (interquartile range) for dialysis vintage and mean (standard deviations) for other continuous data. Arbitrary cut-off values close to the median value of linoleic and arachidonic acid were used to define groups. Differences between groups were evaluated using Chi-square for categorical data, Mann-Whitney U-test for dialysis vintage and Students t-test for other continuous data.

**Table 2. Estimated mortality and graft loss risk according to plasma levels of linoleic acid**

| | All-cause mortality | | | Cardiovascular mortality | | |
|---|---|---|---|---|---|---|
| **Model** | HR | 95% CI | P | SHR | 95% CI | p |
| Crude | 0.94 | (0.91, 0.96) | <0.001 | 0.95 | (0.92, 0.99) | 0.02 |
| Adjustment for recipient age and gender | 0.99 | (0.98, 1.01) | 0.47 | 0.99 | (0.95, 1.04) | 0.79 |
| Multivariable adjustment | 0.99 | (0.96, 1.01) | 0.28 | 0.99 | (0.95, 1.04) | 0.79 |
| | **Overall graft loss** | | | **Death censored graft loss** | | |
| **Model** | HR | 95% CI | p | SHR | 95% CI | p |
| Crude | 0.96 | (0.94, 0.98) | <0.001 | 1.01 | (0.98, 1.05) | 0.44 |
| Adjustment for recipient age and gender | 0.99 | (0.97, 1.01) | 0.25 | 0.99 | (0.96, 1.02) | 0.54 |
| Multivariable adjustment | 0.99 | (0.97, 1.01) | 0.26 | 0.99 | (0.96, 1.03) | 0.81 |

Estimated relative risk of reaching all-cause mortality and overall graft loss end-points according to linoleic acid levels using multivariate Cox proportional hazard regression and relative risk of reaching death censored graft loss and cardiovascular mortality end-points using multivariable subdistributional hazard regression adjusting for the competing risks of recipient death (death censored graft loss) or death from non-cardiovascular causes (cardiovascular mortality).

Candidate variables in multivariable survival analysis registered at time of transplantation: Recipient age and gender, donor age, transplant era (date of transplantation prior to or after 1st January 2007), atherosclerotic disease (coronary artery, cerebrovascular and/or peripheral vascular disease), diabetes mellitus, smoking status (current smoker, former smoker or life-long non-smoker), dialysis vintage (time in dialysis prior to kidney transplantation), preemptive transplantation (no dialysis therapy before kidney transplantation), first or previous renal transplant, donor status (living or deceased) and number of human leukocyte antigen DR mismatches. Candidate variables registered at 10 weeks post-transplant: Choice of calcineurin inhibitor, body mass index, plasma cholesterol, serum albumin, estimated glomerular filtration rate and use of antihypertensive drugs.

**Table 3. Associations between plasma levels of linoleic acid (panel A) and arachidonic acid (Panel B) and cardiovascular risk markers in renal transplant recipients**

## Panel A. Linoleic acid

**Age and gender adjusted linear regression analysis**

| Cardiovascular risk markers | n | Unstd. β-coeff. (95% CI) | Std. β-coeff. | *p* | $R^2$ |
|---|---|---|---|---|---|
| Resting heart rate, *bpm* | 739 | 0.32 (0.04, 0.60) | 0.09 | 0.02 | 0.02 |
| Systolic blood pressure, *mmHg* | 741 | 0.08 (-0.30, 0.46) | 0.02 | 0.67 | 0.14 |
| Diastolic blood pressure, *mmHg* | 741 | -0.07 (-0.32, 0.18) | -0.02 | 0.59 | 0.04 |
| Pulse wave velocity, *m/sec* | 757 | 0.04 (-0.02, 0.10) | 0.04 | 0.20 | 0.31 |
| Triglycerides, *mg/dL* | 1978 | -1.29 (-2.81, 0.22) | -0.04 | 0.09 | 0.01 |
| HDL cholesterol, *mg/dL* | 1982 | 0.50 (0.25, 0.75) | 0.09 | <0.001 | 0.07 |
| LDL cholesterol, *mg/dL* | 1961 | 0.02 (-0.91, 0.96) | 0.01 | 0.96 | 0.02 |
| Fasting plasma glucose, *mg/dL* | 1641 | -0.27 (-0.52, -0.03) | -0.05 | 0.03 | 0.04 |
| 2 hr post-challenge plasma glucose, *mg/dL* | 1637 | -0.84 (-1.48, -0.19) | -0.06 | 0.01 | 0.06 |

**Multivariable linear regression analysis**

| Cardiovascular risk markers | n | Unstd. β-coeff. (95% CI) | Std. β-coeff. | *p* | $R^2$ |
|---|---|---|---|---|---|
| Resting heart rate, *bpm* | 709 | 0.11 (-0.16, 0.39) | 0.03 | 0.42 | 0.10 |
| Systolic blood pressure, *mmHg* | 711 | 0.22 (-0.15, 0.59) | 0.04 | 0.24 | 0.21 |
| Diastolic blood pressure, *mmHg* | 711 | 0.11 (-0.14, 0.36) | 0.03 | 0.38 | 0.13 |
| Pulse wave velocity, *m/sec* | 727 | 0.01 (-0.05, 0.07) | 0.01 | 0.77 | 0.39 |
| Triglycerides, *mg/dL* | 1948 | -0.51 (-2.03, 1.02) | -0.02 | 0.52 | 0.07 |
| HDL cholesterol, *mg/dL* | 1952 | 0.42 (0.18, 0.67) | 0.08 | 0.001 | 0.14 |
| LDL cholesterol, *mg/dL* | 1931 | 0.59 (-0.37, 1.56) | 0.03 | 0.23 | 0.06 |
| Fasting plasma glucose, *mg/dL* | 1611 | -0.31 (-0.54, -0.07) | -0.06 | 0.01 | 0.15 |
| 2 hr post-challenge plasma glucose, *mg/dL* | 1607 | -0.71 (1.36, -0.06) | -0.05 | 0.03 | 0.10 |

## Panel B. Arachidonic acid

**Age and gender adjusted linear regression analysis**

| Cardiovascular risk markers | n | Unstd. β-coeff. (95% CI) | Std. β-coeff. | *p* | $R^2$ |
|---|---|---|---|---|---|
| Resting heart rate, *bpm* | 739 | 0.25 (-0.24, 0.76) | 0.04 | 0.32 | 0.02 |
| Systolic blood pressure, *mmHg* | 741 | -0.01 (-0.69, 0.68) | -0.01 | 0.99 | 0.14 |
| Diastolic blood pressure, *mmHg* | 741 | -0.41 (-0.87, 0.05) | -0.06 | 0.08 | 0.04 |
| Pulse wave velocity, *m/sec* | 757 | 0.14 (0.03, 0.26) | 0.08 | 0.01 | 0.32 |
| Triglycerides, *mg/dL* | 1978 | 1.85 (-1.06, 4.75) | 0.03 | 0.21 | 0.01 |
| HDL cholesterol, *mg/dL* | 1982 | -1.34 (-1.81, -0.86) | -0.12 | <0.001 | 0.07 |
| LDL cholesterol, *mg/dL* | 1961 | -1.33 (-3.13, 0.46) | -0.03 | 0.15 | 0.02 |
| Fasting plasma glucose, *mg/dL* | 1641 | 1.31 (0.84, 1.77) | 0.13 | <0.001 | 0.05 |
| 2 hr post-challenge plasma glucose, *g/dL* | 1637 | 2.76 (1.52, 4.00) | 0.11 | <0.001 | 0.07 |

**Multivariable linear regression analysis**

| Cardiovascular risk markers | n | Unstd. β-coeff. (95% CI) | Std. β-coeff. | *p* | $R^2$ |
|---|---|---|---|---|---|
| Resting heart rate, *bpm* | 709 | 0.38 (-0.12, 0.88) | 0.05 | 0.14 | 0.10 |
| Systolic blood pressure, *mmHg* | 711 | -0.32 (-1.01, 0.35) | -0.03 | 0.35 | 0.21 |
| Diastolic blood pressure, *mmHg* | 711 | -0.30 (-0.75, 0.15) | -0.05 | 0.19 | 0.13 |
| Pulse wave velocity, *m/sec* | 727 | 0.10 (-0.02, 0.21) | 0.05 | 0.09 | 0.39 |
| Triglycerides, *mg/dL* | 1948 | -0.31 (-3.28, 2.65) | -0.01 | 0.84 | 0.07 |
| HDL cholesterol, *mg/dL* | 1952 | -1.04 (-1.52, -0.56) | -0.09 | <0.001 | 0.14 |
| LDL cholesterol, *mg/dL* | 1931 | -1.62 (-3.48, 0.23) | -0.04 | 0.09 | 0.06 |
| Fasting plasma glucose, *mg/dL* | 1611 | 0.97 (0.51, 1.43) | 0.10 | <0.001 | 0.16 |
| 2 hr post-challenge plasma glucose, *g/dL* | 1607 | 1.89 (0.62, 3.17) | 0.07 | 0.004 | 0.11 |

Age- and gender adjusted and multivariable linear regression. Regression coefficients and adjusted explained variance ($R^2$) for the final model are presented. Candidate variables (p<0.10 for inclusion) are given in the text.

**Table 4. Associations between plasma levels of linoleic acid (panel A) and arachidonic acid (Panel B) and inflammatory markers in renal transplant recipients**

## Panel A. Linoleic acid

| | Age and gender adjusted linear regression analysis | | | | |
|---|---|---|---|---|---|
| **Inflammatory markers** | **n** | **Unstd. β-coeff. (95% CI)** | **Std. β-coeff.** | ***p*** | **$R^2$** |
| GDF15, *ng/L* | 838 | 0.99 (0.98, 1.00) | -0.05 | 0.10 | 0.23 |
| PTX3, *ng/mL* | 838 | 0.00 (0.98, 1.00) | -0.05 | 0.13 | 0.03 |
| sTNFR1, *ng/mL* | 832 | 1.00 (0.99, 1.01) | 0.001 | 0.97 | 0.06 |
| Chemerin, *ng/mg* | 836 | -1.42 (-2.68, -0.17) | -0.08 | 0.03 | 0.04 |
| IL-6, *ng/mL* | 765 | 1.01 (0.98, 1.04) | 0.03 | 0.39 | 0.04 |
| IL-10, *ng/mL* | 813 | 1.02 (1.00, 1.04) | 0.07 | 0.06 | 0.01 |

| | Multivariable linear regression analysis | | | | |
|---|---|---|---|---|---|
| **Inflammatory markers** | **n** | **Unstd. β-coeff. (95% CI)** | **Std. β-coeff.** | ***p*** | **$R^2$** |
| GDF15, *ng/L* | 837 | 0.99 (0.98, 1.00) | -0.09 | 0.001 | 0.43 |
| PTX3, *ng/mL* | 837 | 0.99 (0.98, 1.00) | -0.06 | 0.08 | 0.10 |
| sTNFR1, *ng/mL* | 831 | 1.00 (0.99, 1.01) | -0.02 | 0.50 | 0.32 |
| Chemerin, *ng/mg* | 835 | -1.44 (-2.70, -0.20) | -0.08 | 0.02 | 0.08 |
| IL-6, *ng/mL* | 764 | 1.00 (0.98, 1.03) | 0.02 | 0.59 | 0.12 |
| IL-10, *ng/mL* | 812 | 1.26 (0.99, 1.05) | 0.07 | 0.06 | 0.08 |

## Panel B. Arachidonic acid

| | Age and gender adjusted linear regression analysis | | | | |
|---|---|---|---|---|---|
| **Inflammatory markers** | **n** | **Unstd. β-coeff. (95% CI)** | **Std. β-coeff.** | ***p*** | **$R^2$** |
| GDF15, *ng/L* | 838 | 1.03 (1.01, 1.04) | 0.01 | 0.001 | 0.23 |
| PTX3, *ng/mL* | 838 | 1.00 (0.99, 1.02) | 0.02 | 0.61 | 0.03 |
| sTNFR1, *ng/mL* | 832 | 1.01 (1.00, 1.03) | 0.07 | 0.03 | 0.06 |
| Chemerin, *ng/mg* | 836 | 3.07 (0.79, 5.40) | 0.10 | 0.01 | 0.02 |
| IL-6, *ng/mL* | 765 | 1.03 (0.98, 1.08) | 0.04 | 0.23 | 0.04 |
| IL-10, *ng/mL* | 813 | 1.02 (0.97, 1.06) | 0.03 | 0.47 | 0.003 |

| | Multivariable linear regression analysis | | | | |
|---|---|---|---|---|---|
| **Inflammatory markers** | **n** | **Unstd. β-coeff. (95% CI)** | **Std. β-coeff.** | ***p*** | **$R^2$** |
| GDF15, *ng/L* | 837 | 1.02 (1.01, 1.03) | 0.08 | 0.004 | 0.43 |
| PTX3, *ng/mL* | 837 | 1.01 (0.99, 1.03) | 0.04 | 0.25 | 0.10 |
| sTNFR1, *ng/mL* | 831 | 1.01 (1.00, 1.02) | 0.05 | 0.07 | 0.32 |
| Chemerin, *ng/mg* | 835 | 2.37 (0.09, 4.64) | 0.07 | 0.04 | 0.08 |
| IL-6, *ng/mL* | 764 | 1.04 (0.99, 1.09) | 0.05 | 0.15 | 0.12 |
| IL-10, *ng/mL* | 812 | 1.01 (0.97, 1.06) | 0.02 | 0.57 | 0.08 |

Age- and gender adjusted and multivariable linear regression. Regression coefficients and adjusted explained variance ($R^2$) for the final model are presented. Candidate variables ($p<0.10$ for inclusion) are given in the text.

Abbreviations: *GDF15*: Growth differentiation factor 15. *PTX3*: Pentraxin-3. *sTNFR1*: Soluble tumor necrosis factor receptor 1. *IL-6*: Interleukin 6. *IL-10*: Interleukin 10.