Lifestyle, Inflammation, and Vascular Calcification in Kidney Transplant Recipients
Sotomayor, Camilo G.

DOI: 10.33612/diss.135859726

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date: 2020

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Chapter 9

Lifestyle, Inflammation, and Vascular Calcification in Kidney Transplant Recipients: Perspectives on Long-Term Outcomes
the role of lifestyle-related factors including diet and exposure to toxic contaminants is an underexplored area of investigation in the kidney transplantation field.\textsuperscript{1\textendash}6 Beyond hazards of immunological nature, a systematic assessment of potentially modifiable —yet rather overlooked— risk factors for the long-standing high risk of graft failure beyond the first-year post-transplant and on the excess cardiovascular risk of kidney transplant recipients (KTR), may reveal novel targets for clinical intervention to optimize long-term health and downturn current rates of premature death. It should also be taken into account that while kidney transplantation aims to restore kidney function, it incompletely mitigates mechanisms of disease such as chronic low-grade inflammation with persistent redox imbalance, and deregulated mineral and bone metabolism.\textsuperscript{7\textendash}26 In addition to pro-inflammatory effects of various degrees of uraemia, a chronic low-grade immunologic response to the kidney allograft, and the unavoidable long-term toxicity of maintenance immunosuppressive therapy further feed and perpetuate these mechanisms of disease.\textsuperscript{12,13,27\textendash}43 Remarkably, while the vicious circle between inflammation and oxidative stress as common final pathway of a multitude of insults plays an established pathological role in native chronic kidney disease (CKD), its characterization post-kidney transplant has been less than satisfactory. Next to a chronic inflammatory status, markedly accelerated vascular calcification persists after kidney transplantation, and is likewise suggested a major independent mechanism of which its mitigation may counterbalance the excess risk of cardiovascular disease post-kidney transplant.\textsuperscript{17,23,44}

The aim of this thesis was two-fold. First, we aimed to assess whether modifiable dietary elements and toxic environmental contaminants may explain increased risk of cardiovascular mortality and late graft failure in KTR. Next, we aimed to investigate specific laboratory and clinical readouts with a proposed role within persisting mechanisms of disease post-kidney transplantation (\textit{i.e.}, inflammation and redox imbalance, and vascular calcification), as potential non-traditional risk factors for adverse long-term outcomes in KTR.
SUMMARY

Part I — Lifestyle; Healthy Diet & Toxic Contaminants

In chapter 2 we analyzed the association and interaction of fruits and vegetables consumption with estimated glomerular filtration rate (eGFR) and proteinuria on risk of cardiovascular and overall mortality in a population of kidney transplant recipients (KTR) with a functioning graft ≥1 year. In this study, we found that relatively higher vegetable consumption (e.g. a relative increase of 1 tablespoon of vegetables per day) is strongly associated with lower risk of cardiovascular and overall mortality in KTR (~50% and ~25% decreased risk, respectively). Noteworthy, this association was independent of socioeconomic status, physical activity, traditional cardiovascular risk factors, eGFR and proteinuria. Vegetables contain fiber, vitamin A, vitamin C, vitamin K, and phytochemicals such as carotenoids and the broad group of polyphenols including phenolic acids and flavonoids. Indeed, the inverse association between vegetable consumption and cardiovascular risk has largely and consistently been reported in epidemiological studies over general population.

In the clinical setting of pre-transplant end-stage renal disease (ESRD), patients have long been advised to avoid fruit and vegetables consumption to limit potassium intake, and there is no clear clinical incentive — likely due to lack of evidence — to prescribe liberation of fruits and vegetables consumption post-kidney transplant. Indeed, underscoring the claim that systematic assessment is needed to accurately translate a priori benefits of a diet higher in fruits and vegetables to CKD patients, we found an independent inverse association of fruit consumption on risk of cardiovascular mortality, although particularly within KTR with eGFR >45 mL/min/1.73 m² or absence of proteinuria.

It should be noted, nevertheless, that within the subgroup of KTR with lower eGFR or with proteinuria, a higher fruit consumption did not associate with increased mortality risk. This is a finding of paramount relevance. KTR are patients that have been largely advised and trained to avoid fruits and vegetables because these food items tend to have the highest concentration of potassium, which may lead to an increased risk of hyperkalaemia as eGFR declines, which is, in turn, a potentially life-threatening condition because of the associated risk of ventricular arrhythmia and cardiac arrest. However,
it has been observed that rich-potassium plant-based diets have not shown to induce hyperkalemia in CKD patients, likely due to a concomitant high-fiber content that facilitates gastrointestinal transition, allowing less potassium to be absorbed.\textsuperscript{64,68–73} Moreover, the health benefits of fruits and vegetables rich in potassium may be related to their alkalinizing effects, as supported by previously observed reductions in metabolic acidosis, even in nondiabetic CKD patients with eGFR 15–29 mL/min/1.73 m\textsuperscript{2}.\textsuperscript{64,70,73,74} Remarkably, accumulating evidence shows that vegetable-based diets may rather have pleiotropic beneficial effects for CKD patients (Figure 1).\textsuperscript{64}

**Figure 1.** Pleiotropic beneficial effects of a vegetable-based diet. This scheme shows that a vegetable-based diet may yield multi-fold health benefits through a direct nutritional contribution and through changes in the intestinal microbiota. Adapted from “Vegetable-based diets for chronic kidney disease? It is time to reconsider” by Cases A, *Nutrients* 2019, 11, 1263.

Our findings in this chapter are agreement with recent studies suggesting that patients with decreased kidney function may overall benefit from higher consumption of fruits and vegetables rich in potassium.\textsuperscript{70,75,76} We provide the first evidence to pave the way towards recommendations that support
an overall survival benefit of a relative increase of fruits and vegetables consumption post-kidney transplantation. We remark, however, that caution may still be needed with patients with eGFR <30 mL/min/1.73 m², because for this particular patients, a potassium-restrictive diet is still suggested be the goal until pending data may support otherwise.73 Further investigation is warranted to guarantee the safety of a diet richer in fruits and vegetables in KTR, and to evaluate whether the benefit of fruit and vegetable consumption has no upper limit as it is considered to be in the general population.77

In chapter 3 we aimed to investigate the potential inverse association of fish intake with risk of cardiovascular and overall mortality in KTR. Fish are rich in the omega-3 polyunsaturated fatty acids (n-3 PUFA) EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), which are nutrients which have been suggested to improve cardiovascular health.78–83 Proposed beneficial health effects of marine-derived n-3 PUFA are wide-ranging, and may be favourably impacting inflammation, fibrosis, lipid modulation, plaque stabilization, blood pressure, artery calcification processes, and endothelial function.84–95 Each of these properties may separately and synergestically underlie, to a certain extent, the inverse association between marine-derived n-3 PUFA intake, or fish intake, and cardiovascular mortality risk observed in this cohort of Dutch KTR.

Our consistent findings on the association of either n-3 PUFA or fish intake are relevant because current evidence derived from interventional studies based on isolated supplementation of EPA-DHA with the aim of decreasing risk of cardiovascular mortality in KTR is rather controversial or yet insufficient to validate its clinical uptake and prescription.96,97 Importantly, it should be realized that the relation between EPA-DHA intake and intermediate cardiovascular endpoints is the most steep within typical Western levels of intake (<750 mg/d EPA-DHA), with only very little additional benefit reached by supplementary doses.87,88,98–107 With the exception of triglyceride-lowering effects, beyond typical dietary doses, EPA-DHA supplementation rather seems to reach a plateau on favourably effects on modulation of myocardial sodium and calcium ion channels, reducing susceptibility to ischemia-induced arrhythmia, reduced left ventricular workload and improved myocardial efficiency as a result of reduced heart rate, lower systemic vascular resistance, and improved diastolic filling (Figure 2).87,88,98–107
Figure 2. Dose-response and time-course of the effects of EPA-DHA intake on intermediate cardiovascular endpoints. This scheme shows that at typical Western levels of EPA-DHA intake, the observed dose-response relation is the steepest for most intermediate cardiovascular endpoints. Thereafter, upon supplementation, a subsequent plateau is generally observed, with exception of the dose-response relation on triglyceride-lowering effect. Potentially important effects on endothelial, autonomic, and inflammatory responses are not shown because dose responses and time courses of such effects on clinical risk are not well established.\textsuperscript{108–110} Figure reprinted from “Fish Intake, Contaminants, and Human Health” by Mozaffarian D,\textsuperscript{JAMA} 2006, 296, 1885–1889.

Fish is the main dietary source of EPA-DHA, and its consumption may provide EPA-DHA to cover the dose that favorably impacts cardiovascular risk the most. Its inclusion in diet of KTR seems reasonable because fish is a good source of protein without the accompanying ingestion of high amounts of saturated fat as is the case with fatty meat products. In our study population, KTR in the highest category of fish intake (≥15 g/day) had a median (interquartile range) intake of EPA-DHA [240 (170–334) mg/d], which is noticeably lower than the upper limit of dietary intake that yields the most beneficial impact on cardiovascular risk. This observation may suggest that fish intake in Dutch KTR is relatively low, pointing towards a large extent overlooked, cost-effective, and patient-centered risk management strategy to decrease cardiovascular mortality post-kidney transplant. Furthermore, these findings underscore that prior to the implementation of any potential
supplementary strategy, recommendations may be aimed to reaching typical doses of EPA-DHA dietary intake. In this regard, it is first important to know that the quantity of servings needed to reach a modest consumption of EPA-DHA (~250 mg per day on average) varies upon particular fish species. In agreement with most dietary guidelines, it is estimated that ~1-2 servings/week of fatty (oily or dark meat) fish (e.g., anchovies, herring, salmon, sardines, trout, white tuna) provide enough EPA-DHA, while somewhat higher numbers of servings per week are recommended if lean (white meat) fish is consumed.111,112

Controversy arises, however, because dietary fish intake also represents the major source of human exposure to organic mercury.113–118 Indeed, in agreement with previous studies, we found that circulating mercury concentrations increased with greater fish consumption.119–121 Hereafter, in adults, the main concern is the potentially detrimental effect of chronic low-level mercury exposure — from modest fish consumption — on cardiovascular risk and outcomes.122 Both systemic (indirect) mechanisms and direct cardiovascular effects of mercury have been described.123–126 In this study of Dutch KTR, we observed mercury concentrations comparable to, yet qualitatively lower than, previous reports within the European area and in the United States.127–129 We found no clear evidence for a counteracting effect by circulating mercury concentrations on the association between fish intake and the risks of cardiovascular and overall mortality. Yet, because observed circulating mercury values were within normal range (<5 μg/L), we cannot exclude the possibility that such counteracting effect could become perceptible over higher concentrations, or apparent by instead controlling for mercury levels in toenails or hair, which are biomarkers that may better provide an assessment of long-term mercury exposure.118,130,131 It should be realized, however, that while intermediate cardiovascular effects of mercury are suggestive, results on the impact on human cardiovascular disease are rather conflicting.132–136

In this regard, it should be noticed that fish is also rich in selenium, an essential trace element that may protect against both cardiovascular disease and toxic effects of mercury.137 The latter may potentially explain that coefficient estimates of the protective effect of fish intake were qualitatively better than those observed for n-3 PUFA intake alone. These data are in agreement with dietary guidelines supporting fish consumption as essential element of a healthy diet, with a potentially large public health impact from even small increases in fish consumption, and provides the first observational
evidence to aid on the development of cautious approaches for the nutritional management of patients post-kidney transplantation.53 Overall, these findings underscore that investigation of interventional strategies based on individualized recommendations to increase fruit, vegetable, and fish intake in KTR is warranted to substantiate these observational associations, and thus inform practice and policy.

Beyond fish and seafood-derived mercury pollution, cadmium is another environmental and lifestyle-related toxic contaminant that may theoretically be particularly hazardous in the post-kidney transplant setting, on the basis that in such long-term oxidative stress settings, cadmium-induced nephrotoxicity has been shown to associate with impaired kidney function at concentrations that are otherwise considered non-toxic.138–140 For the first time in KTR, in chapter 4 we provided data to suggest that nephrotoxic exposure to cadmium represents an as yet overlooked hazard for preserved graft functioning. We demonstrated that higher plasma cadmium is independently and consistently associated with increased risk of late graft failure and kidney function decline in KTR. The study methods we used and further findings we did warrant the following remarks.

First, it should be realized that the kidney proximal tubule, which is known to be the most affected by cadmium, is not reached by whole blood, but plasma ultra-filtrate. Our study is unique in relation to previous literature in that it analysed the likely most suitable sample — that is, all circulating cadmium except the type bound to erythrocytes — to study the potential impact of circulating cadmium as a proxy for exposure on endpoints of kidney function. Second, only 2 patients reached concentrations of plasma cadmium that are currently considered in the toxic range, yet even with these plasma cadmium distribution outliers excluded, we found a strong dose-response association with risk of late kidney graft failure. This finding is in agreement with the notion that cadmium is hazardous for kidney health ranging from even small levels of exposure, which highlights that bodily cadmium is a topic of clinical concern and may be in need of guidance to avoid exposure, appropriate monitoring and timely management.141–146 Third, providing pathophysiological support to the main finding, as well as interesting data for clinical outpatient follow-up of KTR, we showed that cadmium may be particularly hazardous in patients with abnormal liver enzyme levels. This observation is agreement with the understanding that once absorbed, cadmium is temporarily stored in the liver (bound to metallothionein). Thereafter, upon hepatocytes turnover, cadmium-
metallothionein is released into the circulation, filtered at the glomerulus, and reabsorbed at the proximal tubule, where it builds up with a half-life of up to 45 years.\textsuperscript{147} The latter, once again emphasizes the relevance of minimizing exposure to cadmium, which is indeed the most important therapeutic measure to prevent chronic toxicity. In this regard, and in agreement with previous literature, our findings support that smoking is a significant, yet modifiable, source of exposure to cadmium.

Whereas there is no specific therapy for cadmium-associated CKD, non-toxic cadmium-chelation may be feasible. Calcium ethylenediaminetetraacetic acid chelation of lead, which is a heavy metal with comparable nephrotoxicity to cadmium, has demonstrated to slow progression of ESRD.\textsuperscript{148–150} Our findings underscore that outpatient cadmium monitoring and cadmium-targeted interventional approaches may represent novel and meaningful risk-management strategies to decrease the burden of late kidney graft failure.

**Part II — Inflammation and Oxidative Stress & Vascular Calcification**

Traditional cardiovascular risk factors do not suffice to account for the excess cardiovascular risk of KTR. The long-standing interplay and feedback loop between inflammation and oxidative stress — due to both residual kidney function loss and \textit{de novo} transplant milieu-specific agents — provides a theoretical and conceptual framework upon which upcoming research may deepen the understanding of the pathophysiological status of KTR once they reach an otherwise stable clinical stage. Cutting-edge evidence on the potential hazard of novel (\textit{non-traditional}) cardiovascular risk factors post-kidney transplant may aid on explaining excess cardiovascular risk, and potentially subsidize the development of novel therapeutic strategies.

In chapter 5, we first approached the study of overall patients’ survival in relation to vitamin C status. We measured plasma concentrations of the anti-inflammatory and anti-oxidant agent vitamin C in stable KTR, and evaluated the prevalence of patients with plasma levels within the range of depletion (≤28 µmol/L or 0.5 mg/dL) in order to assess its potential association with risk of mortality. We found that vitamin C depletion was common (22%) in a stable outpatient population of KTR, and independently associated with an almost two-fold increased risk of overall mortality. In line, we observed an approximately 25% decreased risk of mortality per doubling of plasma vitamin C concentration. Because we were interested in exploring the potential underlying involvement of inflammation, and to give pathophysiological
support to our findings, we furthermore studied the potential mediation effect of predefined inflammatory biomarkers, including high-sensitivity C-reactive protein, soluble intercellular cell adhesion molecule 1, and soluble vascular cell adhesion molecule 1. These biomarkers were used to compute a composite inflammatory score to allow an overall characterisation of the inflammatory status. This scoring procedure has the advantage of reducing the influence of measurement error and biological variability and avoids the problem of multiple testing in analyses performed with each biomarker separately. We found that this composite score of inflammatory biomarkers approximately explained one-third of the association of vitamin C with mortality risk. These results support the notion that the beneficial effect of vitamin C on patients’ survival occurs, at least to a considerable extent, through decreasing the chronic low-grade inflammatory status of KTR, and underscore its sizeable relevance as novel risk factor for premature mortality post-kidney transplantation.

In this regard, it should be realized that previous studies in haemodialysis patients have shown that vitamin C supplementation is effective in decreasing inflammatory biomarkers. Whether such effect of vitamin C-based interventional strategies leads to an impact on long-term risk of mortality post-kidney transplant warrants further studies. It is important to remark that, to the best of our knowledge, previous studies (in different clinical settings) have performed randomized supplementation of fixed doses of vitamin C, despite initial vitamin C status. This is relevant because the therapeutical potential in non-depleted patients may be relatively low compared to patients with sub-physiological vitamin C status, and vitamin C deficient patients may need even higher supplementation doses to reach physiological levels and make apparent the benefits from intervention. Although moderate doses of vitamin C supplementation (up to 1 g/d) are considered safe, the former point is relevant because vitamin C supplementation is not exempt of potential drawbacks such as oxalosis and toxicity, of which its appearance would largely depend on initial (pre-intervention) vitamin C status. While suggested vitamin C intake (40 mg/d for adults) can be obtained from a healthy diet, future investigations aimed to explore the potential of pharmacological interventions, are therefore suggested be designed to (i) take into account initial vitamin C status, (ii) consider individualized vitamin C supplementation, and (iii) monitor vitamin C status in order to adhere to reference values (28–85 µmol/L or 0.5–1.5 mg/dL).

To allow a more comprehensive study of the interplay between inflammation
and oxidative stress post-kidney transplantation, and to evaluate its postulated significance as non-traditional factor partially explaining excess cardiovascular risk of KTR, in chapter 6 we measured circulating levels of two specific oxidative stress biomarkers that —through known intracellular signalling pathways— lead to up-regulation of inflammatory responses and amplify oxidative stress. The results of this study firstly support the epidemiological relevance of cardiovascular disease as leading cause of mortality in KTR, by showing that 52% of deaths that occurred during ~7 years of follow-up were due to cardiovascular causes.

Next, we consistently observed that per standard deviation relative increment of the oxidative stress biomarkers under study, i.e., \( \tilde{N}^\epsilon-(\text{Carboxymethyl}) \) lysine and \( \tilde{N}^\epsilon-(\text{Carboxyethyl}) \) lysine (advanced glycation endproducts, AGE), patients were at a clinically meaningful ~50% increased risk of cardiovascular mortality post-kidney transplant. Remarkably, these prospective associations were independent of eGFR, proteinuria and traditional cardiovascular risk factors such as body mass index, diabetes, blood pressure and smoking status.

Furthermore, we found that free thiol groups and soluble vascular cell adhesion molecule-1 consistently explained ~35% of the association between higher levels of AGE and increased risk of cardiovascular mortality. Our results may support the hypothesis that —upon binding to specific cellular receptors— AGE may up-regulate downstream biomarkers of inflammation and oxidative stress. These findings support the notion that inflammation and endothelial dysfunction are redox-sensitive responses, and that intracellular pathways involving activation of transcription factors ultimately feed the loop between oxidative stress and inflammatory responses, contributing to excess cardiovascular risk post-kidney transplant.

Dietary AGE content is also an important contributor to AGE accumulation in CKD patients. Future investigations are warranted to evaluate whether interventions aimed at decreasing exogenous dietary sources of AGE may decrease oxidative stress and inflammation, and represent safe and cost-effective cardiovascular risk-management strategies.\textsuperscript{160–162} Furthermore, with diverse pharmacological agents aimed at inhibiting the formation of AGE, and with novel AGE breakers increasingly becoming available,\textsuperscript{163} these findings highlight the need of performing external validation of our results in different and larger populations of KTR to further support exploration of potential novel avenues of AGE-targeted interventions to decrease the burden of premature cardiovascular mortality in KTR.
Inflammation also plays a cornerstone signalling role linking challenges of both immune and non-immune nature with interstitial fibrosis and tubular atrophy, which represents a common pathological pathway to mechanisms leading to kidney injury.\textsuperscript{33,34,43,164–166} This concept came to set out the basis for the hypothesis that structural damage and detrimental function of the kidney are the cumulative consequence of a variety of hazards associated with up-regulation of inflammatory status and pro-oxidant responses as shared response.\textsuperscript{33,34,43,166} In chapter 7 we studied galectin-3, which is a $\beta$-galactoside-binding lectin and novel biomarker of acute and chronic low-grade inflammation, previously shown to be involved in mechanisms leading to kidney fibrosis, and most recently linked with increased risk of incident CKD. For the first time, our study shows that galectin-3 levels are remarkably high in KTR, and independently associated with increased risk of graft failure over $\sim$10-years of follow-up, with particularly strong associations among KTR with smoking history or with high systolic blood pressure. The observed interaction with blood pressure is consistent with findings of previous studies in the general population, and may further support the involvement of galectin-3 in postulated novel mechanisms of cardiac-renal interactions. These results complement previous studies on the association of galectin-3 with incident CKD, by extending those to a specific high-risk population of progressive loss of kidney function.\textsuperscript{167,168}

Because galectin-3 has been consistently implicated in the development of fibrosis resulting from inflammatory or toxic insults, it seems compelling to speculate that galectin-3–targeted pharmacological strategies may provide a therapeutic alternative to downturn the deleterious effect of a broad variety of hazards associated with kidney fibrosis and function loss. Such interventional strategies may \textit{a priori} seem particularly promising in the post-kidney transplant clinical setting. Our findings point towards yet-to-unfold opportunities to aid on non-invasive, early and individualized long-term clinical follow-up post-kidney transplant. Prior to the formal proposal of therapeutic potential, however, further investigations on the evolving and broad profile of homeostatic and pathophysiological bioactivities of galectin-3 are warranted.\textsuperscript{169}

Beyond inflammation, a recent elegant study supports the hypothesis that decline of cardiovascular risk post-kidney transplant partly depends on the resolution of chronic kidney disease-mineral and bone disorders (CKD-MBD).\textsuperscript{23} Within the context of CKD-MBD, vascular calcification
— a currently established cardiovascular risk factor in KTR, as shown in a previous study from our group and studies of others — is linked with bone disease due to inter-related pathophysiological mechanisms, setting out the basis for a bone-vascular axis hypothesis. Because disturbed bone and mineral metabolism persists after kidney transplantation, and because maintenance immunosuppressive therapy adds a transplant-specific hazard for bone disease, in chapter 8 we hypothesized that bone mineral density (BMD) is independently and inversely associated with risk of vascular calcification in KTR. Recommendations for BMD testing after transplantation by means of dual-energy X-ray absorptiometry (DXA) have been formally incorporated in the KDIGO 2017 clinical practice guidelines. This imaging technique also allows assessment of abdominal aortic calcification (AAC) with a low radiation burden. Therefore, in this study we expanded the clinical accountability of a DXA scan, by showing that BMD disorders according to DXA scan data are highly prevalent in KTR (54%), and also showing its independent association with increased risk of AAC, thus providing data in favor of the bone-vascular axis hypothesis in KTR. Because DXA scans are non-invasive, relatively accurate and cost-effective, these results underscore the notion that DXA scan is an interesting imaging method for screening of bone mass and vascular calcification early post-kidney transplant.

Because kidney transplantation aims to restore kidney function but incompletely mitigates collateral mechanisms of disease, such as chronic low-grade inflammation with persistent redox imbalance, and deregulated mineral and bone metabolism, the second part of this thesis investigated specific laboratory and clinical readouts with a proposed involvement in such pathological pathways. First, by using the theoretical and conceptual framework of the interplay between inflammation and oxidative stress, we deepened the understanding of the pathophysiological status of KTR once they reach an otherwise stable clinical stage. We were able to assess the contribution of this phenomenon to excess risk of cardiovascular mortality and late kidney graft failure, and point towards potential interventional strategies. Finally, we provided evidence that may support the existence of a bone-vascular axis post-kidney transplant. Future studies are warranted to evaluate whether incorporation of BMD and vascular calcification assessment by means of a DXA scan within the context of outpatient follow-up, may aid on the evaluation and guidance of therapeutic management of CKD-MBD and cardiovascular risk post-kidney transplant.
DISCUSSION AND FUTURE PERSPECTIVES

Kidney transplantation is the preferred treatment for ESRD. In a few decades, advances in immunosuppressive therapy, tissue typing, treatment of infections and surgical techniques led kidney transplantation to evolve from an exotic therapeutic option to gold-standard care. It is known, however, that over recent years, advances in kidney transplantation have been mainly driven by improvements on first-year graft and patient survival. Several challenges remain to be addressed in order to decrease the excessive load of disease post-kidney transplant, and thus ultimately contribute to a longer life-span of kidney transplant recipients (KTR). Future progress in the field of kidney transplantation is expected from amelioration of excess cardiovascular risk and the long-standing burden of late graft failure. This thesis assessed traditional — yet rather overlooked — risk factors post-kidney transplant, and investigated novel (non-traditional) risk factors along the theoretical and conceptual framework in which kidney transplantation aims to restore kidney function but incompletely mitigates mechanisms of disease, which are furthermore perpetuated by the kidney transplantation milieu, ultimately challenging the improvement of long-term outcomes post-kidney transplant. This approach allowed us to underscore potentially modifiable factors, which are suggestive of early, cost-effective and patient-centered interventional strategies, and reflect on future prospects in the field from several perspectives.

From an epidemiological view, we were able to unravel prevailing — yet mostly overlooked — preventive opportunities with a meaningful potential for application at a general clinical level. The inverse associations between fruit, vegetable and fish intake with risk of cardiovascular and overall mortality post-kidney transplantation, point towards promising early risk-management strategies based on practical and cost-effective measures tackling the major cause of premature death with a functioning graft, i.e., cardiovascular mortality. In theory, dietary intervention strategies based on personalized recommendations to increase fruit, vegetable, and fish intake in KTR may yield a meaningful reduction of cardiovascular risk. To the best of our knowledge no clinical trial has been devoted to investigating this hypothesis, despite the fact that the beneficial effect of adequate nutritional control in any chronic disease is universally accepted, and that it is well-recognized that KTR are patients at particularly high cardiovascular risk. Strikingly, currently there is absence of shared agreements on important nutritional aspects post-kidney
transplantation. Indeed, KTR want to hear information on dietary guidelines, because they want to increase their knowledge to have the opportunity to adequately manage their diets and health.\textsuperscript{5,180} Of note, in general kidney disease patients rank dietary research among their priorities.\textsuperscript{181} At difference, clinicians in general consider nutrition of KTR just an additional element of the care planning, which may be reflected by, or due to, the lack of evidence-based recommendations.\textsuperscript{6} Of course, a biased research agenda can have several consequences.\textsuperscript{182} As in other research areas with a mismatch between the amount of published work on different interventions and the degree of interest of consumers or burden of disease, dietary advise and nutritional management of KTR seems to remain an underrepresented problem in the research agenda of the kidney transplantation research community.\textsuperscript{181–183} Considering our findings, investigation of potential interventional strategies based on individualized recommendations to increase fruit, vegetable, and fish intake in KTR is warranted in order to substantiate our observational associations, and thus pave the way to inform practice and policy.

Likewise, although heavy metals are environmental toxins with epidemiological relevance and a demonstrated etiological role on the incidence and progression of CKD, their toxicity continues to receive little to null research priority, which is particularly the case post-kidney transplantation. Available evidence supports the notion that heavy metals are hazardous for kidney health in a dose-dependent fashion, ranging from even small levels of contamination to overt toxicity.\textsuperscript{141–146} These data are consistent with our findings on the association of plasma cadmium with risk of kidney function decline and late kidney graft failure. In our study, cadmium showed a dose-dependent association with graft failure events over increasing tertiles of plasma concentration, which suggests that this is not a matter to be adressed exclusively in highly contaminated regions, but rather a topic of concern over areas with various degrees of cadmium pollution. For the particular case of Europe, it should be taken into account that environmental cadmium pollution during the past century may have caused a fifty-fold rise in its concentration in the human renal cortex, wherein it accumulates 50\% more than in the total kidney, with a half-life of up to 45 years.\textsuperscript{147,184} In The Netherlands and Belgium, moreover, environmental cadmium contamination is of exceptional relevance compared to other countries of the European Union (EU).\textsuperscript{144,185–187} In The Netherlands, the most important origin is transboundary through upstream countries’ accumulating pollution in Dutch sediments and surface water due
to the delta situation of the country. A high cadmium-containing landfill and a large percentage of incinerated household waste may also explain why the cadmium pollution situation in The Netherlands is a special case, being worse than the average EU\textsuperscript{186,187} We suggest that future research be conducted to investigate potentially significant health impact of environmental exposure to cadmium in The Netherlands, with a special focus on longitudinal estimates of kidney accumulation, structural damage, and impaired function, including but not restricted to the particular population of KTR, and potentially also broadly over the general population. Similar recommendations apply to other countries of the EU, and also to other regions of the world wherein heavy metals — particularly cadmium — are strong risk factor candidates to at least partly explain CKD of Uncertain Etiology (CKDu), which currently is a main research goal of the World Health Organisation and the International Society of Nephrology to develop appropriate health policy and public health responses to this issue.\textsuperscript{141,146,188–196} Next, because cadmium reduces insulin levels and has direct cytotoxic effects on the pancreas, liver, adipose tissue and adrenal gland, we also warrant future studies to investigate whether cadmium may synergestically damage the kidney through direct nephrotoxicity and hyperglycemia.\textsuperscript{146,192,197–211} Finally, considering the relevance of abnormal bone mineral density post-kidney transplantation, and reports of previous studies on the association of cadmium exposure with risk of osteoporosis and fracture, future investigations of cadmium in KTR are warranted to evaluate the potential contribution of cadmium exposure to increased risk of abnormal bone mineral density.\textsuperscript{212–220}

At a pathophysiological level we were able to investigate the phenomenon of chronic inflammation with persistent redox imbalance, and provide evidence of the existence of a bone-vascular axis post-kidney transplantation. We propose that these two major mechanisms of disease are incompletely mitigated post-kidney transplantation and may ultimately challenge the improvement of outcomes beyond the first-year post-kidney transplantation and beyond hazards of purely immunological nature. Our data may support the hypothesis that inflammation and oxidative stress, deregulated bone and mineral metabolism, and cardiovascular disease participate in a self-perpetuating cycle, which, if not interrupted, can lead to progressive cardiovascular disease and kidney dysfunction.\textsuperscript{221} These insiduous and rather asymptomatic pathophysiological processes seem to occur over an extended period, from a wide varying time pre-transplantation, further perpetuated and
even promoted within the transplant milieu. Exceedingly high prevalence of cardiovascular disease, precipitated derangements of metabolism as well as high risk of malignancies resemble the general aging process, however, at a particularly accelerated pace in these patients. This consideration may be related to the observation that life-span of KTR has not been positively impacted in parallel with improvements of short-term outcomes, perhaps because advances in immunosuppression, tissue typing, treatment of infections, surgical techniques nor partial recovery of kidney function itself have completely mitigated the aforementioned collateral mechanisms of disease. By characterizing non-traditional risk factors, relevant clinical readouts, and specific signalling agents, this thesis may support the interplay between inflammation and oxidative stress, and deregulated mineral and bone metabolism, as pathways of injury that remain active in seemingly stable KTR and adversely impact long-term outcomes post-kidney transplantation.

Overall, this thesis may support and acknowledge the need of providing a systematic assessment of the impact of traditional risk factors in the specific post-kidney transplant setting, as well as identifying novel and potentially modifiable risk factors potentially explaining excess risk of adverse long-term outcomes in outpatient KTR. On this understanding, our group designed and is currently executing the long-lasting prospective cohort study and biobank of solid organ transplant recipients “TransplantLines”. The general aim of this study is to provide longitudinal data to evaluate the impact of the transplantation milieu on a broad variety of health parameters, and thus generate evidence-based hypotheses for individualized early risk-management strategies to advance survival rates of the transplanted kidney, organ recipient and quality of life after transplantation. Five pillars of data collection (i.e., clinical data, physical tests, cognitive tests, questionnaires, and biomaterials, as depicted in Figure 3) will allow extensive phenotyping of KTR before, during transplantation and at follow-up visits over several years. This multi-fold approach of data collection covering several specialized fields of health care underlines the multidisciplinary efforts needed to comprehensively improve the long-term burden of disease, reduced life-span and impaired quality of life post-kidney transplantation, while at the same time keeping in the center of care each patient in an holistic fashion, and aligning research team’s agenda with the needs of the population it is meant to serve.182,222
Figure 3. Five pillars of data collection and biobanking of TransplantLines. This figure illustrates the multi-fold approach for data collection and biobanking of solid organ transplant recipients, while emphasizing the key centred-role of patients in research and healthcare efforts.

Furthermore, we specially remark on our most recent and ongoing study TransplantLines-Coronary Artery Calcification (CAC), which aims to extend the biobanking efforts by providing imaging examinations and thorough assessment of laboratory parameters specifically aiding to provide an early and non-invasive evaluation of the vascular calcification phenomenon post-kidney transplant, as first step to develop interventions to slow down or arrest the progression of vascular calcification in KTR.

Finally, enclosing the aforementioned perspectives at different levels,
a global research-in-human health point of view may be speculated. The
prevalence of CKD worldwide has steadily increased over recent decades,
in parallel with an ever longer population’s life-span, representing an
unquestionable global public priority.\textsuperscript{223} CKD is an independent risk factor for
cardiovascular disease, of which the risk has also increased in parallel with
longer life expectancy. Cardiovascular disease, moreover, importantly adds to
the worldwide burden of disease, underscoring that the mechanisms of cross-
talk between kidney disease and cardiovascular disease will also escalate in
relevance in the near future. In a population characterized by premature death,
this thesis provides interesting data highlighting the self-perpetuating cycle
between CKD, inflammation with persisting redox imbalance, and deregulated
bone and mineral metabolism, and cardiovascular disease, which is thought
to then further promote loss of kidney function. Previous studies, argue that
—over varying genetic setting— the persistence over time of inflammatory
responses to immune and other stressing stimuli are of importance, and provide
a biologic background favouring susceptibility to age-related chronic diseases,
such as cardiovascular disease, cancer, CKD, osteoporosis and diabetes, with
detrimental effects that ultimate adversely affect life-span.\textsuperscript{224} Future research
aimed at investigating the inter-play between CKD, cardiovascular disease
and other major chronic diseases, particularly within the context of elderly
populations, may be suggested to be based on the theoretical and conceptual
framework of inflammation and oxidative stress, also known as inflamm-
aging.\textsuperscript{224}
CONCLUSION

In the clinical setting of KTR after the first-year post-transplant, in this thesis we studied and characterized the clinical impact of (i) traditional and potentially modifiable —yet rather overlooked— risk factors, such as lifestyle, diet and exposure to toxic contaminants, which are underexplored areas in the kidney transplantation field. This approach unfolded potentially cost-effective and patient-centred opportunities that may increase the life-span of KTR once they reach an otherwise stable clinical stage. Dietary interventional strategies based on individualized recommendations to increase fruit, vegetable, and fish intake in KTR may substantially alleviate the burden of premature death among outpatient KTR. Investigation of the potential impact of policy measures and clinical guidance to decrease the exposure to cadmium and other toxic environmental contaminants is also warranted. Against the background that kidney transplantation aims to restore kidney function but incompletely mitigates collateral mechanisms of disease, this thesis may support the notion that non-traditional risk factors, such as chronic low-grade inflammation with persistent redox imbalance, and deregulated mineral and bone metabolism, may at least partly explain the excess risk of premature death of KTR. Further research on these non-traditional risk factors is also warranted as it may pave the way towards decreasing the long-standing burden of premature death post-kidney transplantation.
REFERENCES


Part II


Summary, Discussion and Future Perspectives


Summary, Discussion and Future Perspectives


91. Christensen JH. Omega-3 polyunsaturated fatty acids and heart rate variability. *Front Physiol* 2011, 2, 84.

102. Mozaffarian D, Gottdiener JS, Siscovick DS. Intake of tuna or other broiled or baked fish versus fried fish and cardiac structure, function, and hemodynamics. *Am J Cardiol* 2006, 97, 216–222.


Part II


Part II


Part II

222. Eisenga MF, Gomes-Neto AW, Van Londen M, et al. Rationale and design of
Part II


