

The Associations of Endotoxemia with Systemic Inflammation Endothelial Activation, and Cardiovascular Outcome in Kidney Transplantation.

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Word Count

Abstract = 286

Main Text = 3436

Running Title

Inflammation and Endothelial Dysfunction in Kidney Transplantation

Funding

Winnie Chan received a research grant from the British Renal Society, and was awarded a PhD research training fellowship from the West Midlands Strategic Health Authority.

List of Abbreviations

CVD.....	Cardiovascular Disease
KTRs.....	Kidney Transplant Recipients
CKD.....	Chronic Kidney Disease
hsCRP.....	High-sensitivity C-Reactive Protein
sE-selectin.....	Soluble E-selectin
ICED.....	Index of Coexistent Disease
Pre-DM.....	Presence of Diabetes Pre-Transplantation
NODAT.....	New Onset Diabetes After Transplantation
SBP.....	Systolic Blood Pressure
DBP.....	Diastolic Blood Pressure
MAP.....	Mean Arterial Pressure
eGFR.....	Estimated Glomerular Filtration Rate
HDL.....	High-Density Lipoprotein
LDL.....	Low-Density Lipoprotein
BMI.....	Body Mass Index
WC.....	Waist Circumference
BCM.....	Body Composition Monitor
LTI.....	Lean Tissue Index
FTI.....	Fat Tissue Index
SD.....	Standard Deviation
IQR.....	Interquartile Range
R.....	Ratio
CI.....	Confidence Interval
LPS.....	Lipopolysaccharides
NO.....	Nitric Oxide

Abstract

Introduction: Cardiovascular disease is the leading cause of death in kidney transplant recipients (KTRs). The prevalence of traditional cardiovascular risk factors is unable to justify the increased incidence of cardiovascular events in KTRs. Inflammation and endothelial dysfunction have recently been identified as potential contributors of such unconventional cardiovascular phenotypes. A potential source of inflammation and endothelial dysfunction in KTRs, may arise through gut derived endotoxemia. The objectives of this study were therefore to investigate the predictors of inflammation and endothelial dysfunction in a prevalent cohort of KTRs.

Methods: This single-centre cross-sectional study enrolled 128 clinically stable KTRs. Fasting serum samples were collected for measurements of high-sensitivity C-reactive protein (hsCRP), soluble E-selectin (sE-selectin), endotoxin, 25-hydroxyvitamin D, adiponectin, uric acid, full lipid-profile, and estimated glomerular filtration rate. Dietary intakes were determined by 3-day food diary. Body composition was measured using bio-impedance based body composition monitor. Central obesity was assessed using waist circumference. Demographic, nutritional and clinical predictors of inflammation (hsCRP) and endothelial function (sE-selectin) were assessed.

Results: Endotoxemia ($R=1.20, p=0.03$), reduced vitamin D ($R=0.82, p=0.04$), high fructose intake ($R=1.12, p<0.001$), decreased dietary fibre intake ($R=0.85, p<0.001$), and increased waist circumference ($R=1.05, p=0.002$) were associated with elevated hsCRP independently. Endotoxemia was also observed to be associated with raised sE-selectin ($R=1.04, p=0.007$) independently of inflammation ($R=1.65, p=0.02$). Other independent predictors of elevated sE-selectin levels include low adiponectin levels ($R=0.96, p=0.004$), increasing waist circumference ($R=1.35, p=0.005$), male ($R=1.07, p=0.01$), and elevated mean arterial pressure ($R=1.35, p=0.006$).

Conclusion: Endotoxemia in KTRs contributes to both systemic inflammation and endothelial dysfunction. The independent association between endotoxemia and endothelial dysfunction suggests a possible non-inflammatory mechanism of endothelial dysfunction. Targeting endotoxemia may serve as a potent upstream intervention for endothelial dysfunction in KTRs, thereby improving cardiovascular outcome in this population.

Introduction

Cardiovascular disease (CVD) is the leading cause of mortality and therefore a major driver to graft loss in kidney transplant recipients (KTRs)¹. Conventional cardiovascular risk factors incompletely explain the increased incidence of cardiovascular events in KTRs², and several studies have highlighted the potential contributions of non-traditional exposures²⁻⁴. Inflammation and activation of the immune system are believed to provoke atherogenesis in the general population⁵, and inflammation correlates with endothelial dysfunction and accelerated atherosclerosis in general⁶ and chronic kidney disease (CKD)⁶⁻⁹ populations. Although less studied in KTRs, recent studies have confirmed inflammation as an important and reproducible risk factor for cardiovascular events, all-cause mortality, and graft failure among KTRs^{2,10-15}.

Despite its undisputed clinical significance, the factors contributing to inflammation among clinically stable KTRs remain under-investigated, and it is unclear whether it is the underlying determinants of inflammation or the inflammatory process itself that leads to such adverse outcomes. Thus far, only one study has reported abdominal obesity and smoking as important modifiable determinants of inflammation among KTRs¹².

Yet recent studies in general and other diseased populations have identified important factors contributing to inflammation and endothelial dysfunction. These include endotoxemia^{16,17}, hypovitaminosis D¹⁸⁻²¹, hyperuricemia²²⁻²⁴, hypoadiponectinemia²⁵⁻²⁷, and high dietary intake of fructose²⁸⁻³⁰. The involvement of such factors in inflammation and endothelial dysfunction

among KTRs remain unexplored, and further investigations are warranted due to its potentially reversible nature, forming targets for future interventions. The objectives of this study were to specifically examine the contribution of these factors in determining post-transplantation inflammation among clinically stable KTRs, and to evaluate the independent associations of inflammation and its causes on circulating markers of endothelial cell damage³¹. It is hoped that the findings from this study will help to define the most appropriate and plausible targets for intervention in this setting.

Methods

Participants and Study Design

KTRs beyond 1 year post-transplantation, with stable graft function (<10% increase in serum creatinine over the preceding 6 months), were recruited to this cross-sectional study between April 2010 and April 2013. Exclusion criteria included episodes of acute rejection within the last 6 months, evidence of sepsis in the last 6 weeks, known active malignancy or chronic infection, history of thyroid disease or adrenal insufficiency, and contraindications for use of bioimpedance-based body composition assessment (implanted or external electronic devices, metallic implants, amputations, pregnancy, and lactation). Of 133 patients approached, 10 did not participate mainly due to work commitment. This study was approved by the local research ethics committee and was conducted in accordance with the principle of Declaration of Helsinki.

Data Collection

Demographic and Clinical Parameters

Age, gender, ethnicity, and time post-transplantation were collected from patients' medical records. Smoking status (never smoked, current, and ex-smoker) and alcohol intake (units per week) were collected by questionnaire. Comorbidity was assessed by Index of Coexistent Disease (ICED) using the algorithm described by the Haemodialysis Study³², with the required data extracted from patients' medical records. In addition, the following clinical parameters were retrieved from patients' medical records: 1) presence of diabetes, either pre-transplantation (pre-DM), or new onset diabetes after transplantation (NODAT); 2) previous acute rejection episodes; 3) dialysis vintage; 4) pre-emptive transplantation; 5) use of statin; and 6) immunosuppressive medication usage, either prednisolone, calcineurin inhibitor, or adjunctive antiproliferative agent.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured semi-recumbent with a fully automatic upper-arm digital blood pressure monitor (Spot Vital Signs LXi; Welch Allyn). Six readings over an 8- to 10- minute period were taken, with the first reading ignored, and the mean of the remaining five used for subsequent derivation of mean arterial pressure (MAP), calculated using the formula $(2DBP+SBP)/3$ ³³.

Laboratory Parameters

Blood samples were taken in the morning following an overnight fast for measurements of urate, 25-Hydroxyvitamin D, estimated glomerular filtration rate (eGFR) derived using four-variable modifications of diet in renal disease equation, and full lipid profile including total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides. Analyses were undertaken in accredited hospital biochemistry laboratory.

hsCRP was measured using a Tina-quant® cardiac C-reactive protein latex high sensitive immunoturbidimetric assay (Roche Diagnostics, Basel, Switzerland). The intra- and inter-assay coefficients of variations were <1.3% and <5.7% respectively.

Adiponectin and sE-selectin were measured using commercially available enzyme-linked immunosorbent assay according to manufacturer's instructions. The intra- and inter-assay coefficients of variation were 3.4% and 5.7% respectively for adiponectin (Linco Ltd, USA)³⁴; and <5% and <10% respectively for sE-selectin (R&D Systems, Germany).

Serum endotoxin was analysed using a commercially available QCL-1000 Limulus Amebocyte Lysate end point assay (Lonza, USA). The intra- and inter-assay coefficients of variation were 3.9% and 9.6% respectively³⁵. The assay has been previously validated.....

Anthropometric Measurements and Body Composition Parameters

Body weight (kg) and height (m) were measured in a standardised procedure with participants wearing light clothing without shoes. Body weight was measured using a digital scale to the nearest 0.01kg. Body height was measured using a stadiometer with the participants standing without shoes and feet together, to the nearest 0.01m. Body mass index (BMI, kg/m²) was calculated as weight (Kg) divided by height squared (m²). Waist circumference (WC, cm) was measured with a non-stretchable standard tape measure, to the nearest 1cm. It was positioned over the unclothed abdomen at the midpoint of the lower thoracic cage and iliac crest in the midaxillary line, as recommended by the World Health Organisation³⁶.

In addition, a well-validated multi-frequency bio-impedance based body composition monitor³⁷ (BCM, Fresenius Medical Care, Germany) was used to assess body composition. Measurements were carried out in a standard manner while the patient was lying supine in a flat and non-conductive bed. The inbuilt physiological body composition model measures whole-body bio-impedance spectroscopy at 50 frequencies (5-1000 kHz) via electrodes placed on the wrist (proximal to the transverse) and ankle (arch on the superior side of the foot). Body composition data including lean tissue index (LTI, kg/m²), fat tissue index (FTI, kg/m²), and volume expansion (%) were displayed after each measurement.

Dietary Intake Parameters

Fructose, dietary fibre, total fat and saturated fat intakes were estimated by a 3-day food diary. Participants were given detailed written instructions on completing an accurate dietary

record for a 3-day period, which included one weekend day, within 1 week before attending the research visit. These instructions were accompanied by verbal explanation from the researcher, which included training in portion size estimation and documentation for both dining in and eating out. The dietary records were reviewed by the researcher for accuracy and completeness at the research visit. Data was entered into Dietplan 6 P3 (Forestfield Software Ltd) nutrition analysis program by the same researcher, avoiding inter-observer variation. Total intakes of all nutrients were calculated by this program.

Statistical Analysis

Statistical analyses were performed using SPSS Statistics 21 (Chicago, IL). Results were presented as mean \pm standard deviation (SD) for normally distributed data or median (interquartile range, IQR) for non-normally distributed data.

Regression diagnostics were performed. Linear regression analysis was used to determine the associations between predictor variables and the continuously distributed outcome variables. The continuously distributed outcome variables with positively skewed distributions underwent logarithmic transformation prior to analysis.

The analyses were performed in two stages. Initially, the effect of each variable was examined in a series of univariate analyses. Subsequently, the joint effect of variables was examined in a multivariate analysis, using a backwards selection procedure to derive the final

model. A type 1 error rate $\leq 5\%$ ($p \leq 0.05$) was considered significant. In the multivariate regression analyses, only the explanatory variables with univariate p -values of < 0.20 were included.

Results

Patient Characteristics

The characteristics of the studied population are shown in **Table 1**.

Determinants of Inflammation (hsCRP) in KTRs

Median serum hsCRP level in this cohort of KTRs was 2.47 (IQR = 1.00-4.89) mg/L. hsCRP levels, (Figure 1a) demonstrated a positively skewed non-gaussian distribution and as such logarithmic transformation was performed prior to regression analyses. A summary of univariate and multivariate regression analyses are shown in Table 2.

When examined individually on univariate analysis, higher hsCRP levels were associated with increased levels of endotoxin (Ratio, $R=1.20$; 95% CI=1.07, 1.34; $p=0.002$), decreased levels of vitamin D ($R=0.67$; 95% CI=0.49, 0.90; $p=0.004$), increasing urate ($R=1.11$; 95% CI=1.00, 1.22; $p=0.007$), increased LDL ($R=1.13$; 95% CI=1.03, 1.23; $p=0.01$), higher

fructose intake (R=1.13; 95% CI=1.12, 1.15; $p<0.001$), lower dietary fibre intake (R=0.85; 95% CI=0.70, 0.90; $p<0.001$), higher saturated fat intake (R=1.14; 95% CI=1.10, 1.18; $p=0.03$), increasing WC (R=1.12; 95% CI=1.06, 1.16; $p<0.001$), and increased FTI (R=1.39; 95% CI=1.23, 1.58; $p<0.001$).

In multivariate analysis, only raised levels of endotoxin (R=1.20; 95% CI=1.08, 1.33; $p=0.03$), decreased levels of vitamin D (R=0.82; 95% CI=0.74, 1.00; $p=0.04$), increased fructose intake (R=1.12; 95% CI=1.09, 1.13; $p<0.001$), decreased dietary fibre (R=0.85; 95% CI=0.79, 1.08; $p<0.001$), whilst an increasing WC (R=1.05; 95% CI=1.02, 1.08; $p=0.002$) were associated with elevated hsCRP independently.

However in KTRs where endotoxin levels reached 2.50EU/mL there was an 50% increase in HsCRP (Figure 1b) As shown in **Figure 1b**, when endotoxin level reaches 2.50EU/mL, an exponential increase in hsCRP was observed. **Figure 1c** presented a negative association between vitamin D and hsCRP levels, but the relationship tailed off when vitamin D levels rose above 50nmol/L. In addition, **Figure 1d** displayed minimal increase in hsCRP with increasing intakes of dietary fructose up to 75g per day; for higher intakes of dietary fructose, a stronger positive relationship was displayed between the two variables. Further, **Figure 1d** indicated a negative association between intakes of dietary fibre and hsCRP levels, but this relationship is only evident when dietary fibre intakes are below 15g per day. Moreover, **Figure 1f** revealed a positive association between WC and hsCRP levels, with a more pronounced increase when WC is greater than 100cm. Of note, a substantial proportion of the variation in inflammation could be explained by these predictor variables ($R^2=67\%$, **Table 2**).

Determinants of Endotoxin Levels in KTRs

In light of the observed relationship between endotoxemia and inflammation, and the lack of available data on the aetiology of endotoxemia in KTRs, a secondary analysis was performed focusing on the factors associated with endotoxemia. Endotoxin levels, shown in **Figure 2a**, demonstrated positively skewed distribution and underwent logarithmic transformation prior to regression analyses. Median serum endotoxin level in this cohort of KTRs was 1.95 (IQR = 1.49-2.38) EU/mL. A summary of univariate and multivariate regression analyses are shown in **Table 3**.

When examined individually on univariate analysis, higher endotoxin levels were associated with lower vitamin D levels ($R=0.82$; 95% CI=0.74, 0.82; $p<0.001$), decreased HDL ($R=0.51$; 95% CI=0.32, 1.22; $p=0.006$), increased LDL ($R=1.67$; 95% CI=1.27, 2.20; $p<0.001$), raised total cholesterol ($R=1.42$; 95% CI=1.12, 1.79; $p=0.004$), higher triglycerides ($R=1.08$; 95% CI=1.06, 1.10; $p<0.001$), increasing fructose intake ($R=1.11$; 95% CI=1.11, 1.22; $p<0.001$), higher intake of saturated fat ($R=1.09$; 95% CI=1.06, 1.12; $p=0.04$), higher WC ($R=1.22$; 95% CI=1.11, 1.49; $p=0.004$), increased FTI ($R=1.65$; 95% CI=1.11, 2.46; $p<0.02$), and decreased LTI ($R=0.89$; 95% CI=0.84, 0.96; $p=0.002$).

However, in the adjusted model, the only significant independent predictors of endotoxemia were decreased vitamin D levels ($R=0.90$; 95% CI=0.82, 0.90; $p<0.001$), higher triglycerides

($R=1.06$; 95% CI=1.04, 1.08; $p<0.001$), higher fructose intake ($R=1.11$; 95% CI=1.00, 1.11; $p=0.01$), and increasing WC ($R=1.22$; 95% CI=1.11, 1.35; $p=0.01$).

As shown in **Figure 2b**, a linear negative association between vitamin D and endotoxin levels was observed, with 10-unit increase in vitamin D levels associated with 9% increase in endotoxin levels. In contrast, **Figure 2c** presented a linear positive association between triglycerides and endotoxin levels, with 1-unit increase in triglycerides associated with 6% increase in endotoxin levels. In addition, **Figure 2d** revealed a positive association between fructose intake and endotoxin levels, but the association is more evident when intakes of fructose are greater than 75g per day. Similarly, as shown in **Figure 2e**, the positive association between WC and endotoxin levels only becomes apparent when WC is higher than 100cm. A borderline effect of reduced LTI ($R=0.95$; 95% CI=0.90, 1.00; $p=0.07$, **Figure 2f**) was found in the final regression model, with a stronger negative association for LTI below 15kg/m^2 . Finally, the associations between endotoxemia with NODAT ($R=1.03$; 95% CI=0.99, 1.06; $p=0.08$) and pre-existing diabetes ($R=1.03$; 95% CI=0.99, 1.08; $p=0.08$) emerged in final model of the multivariate analysis, although statistical significance for both associations were not reached. On average, NODAT and pre-existing diabetes were found to have higher endotoxin levels by 3% in both cases compared with patients without diabetes. Notably, the variables emerged in the final regression model explained 46% of the variation in endotoxin levels ($R^2=46\%$, **Table 3**).

Determinants of Endothelial Function (sE-selectin Levels) in KTRs

Median serum sE-selectin level in this cohort of KTRs were 34.2 (IQR = 24.1-44.8) ng/mL. sE-selectin levels, (**Figure 3a**), demonstrated a positively skewed distribution and a logarithmic transformation was undertaken prior to parametric analysis. **Table 4** summarised the regression analyses pertaining to sE-selectin.

The following predictor variables displayed univariate, unadjusted associations with elevated sE-selectin levels: lower adiponectin levels (R=0.94; 95% CI=0.89, 0.98; $p=0.007$), higher endotoxin levels (R=1.09; 95% CI=1.05, 1.14; $p<0.001$), increasing hsCRP (R=1.49; 95% CI=1.01, 2.22; $p=0.04$), decreased HDL (R=0.93; 95% CI=0.88, 0.99; $p=0.02$), increased triglycerides (R=1.05; 95% CI=1.02, 1.08; $p=0.001$), higher fructose intake (R=1.11; 95% CI=1.00, 1.22; $p=0.04$), increasing WC (R=1.35; 95% CI=1.11, 1.64; $p=0.003$), lower LTI (R=0.98; 95% CI=0.97, 0.99; $p=0.004$), male (R=1.09; 95% CI=1.03, 1.16; $p=0.004$), advancing age (R=1.35; 95% CI=1.11, 1.65; $p=0.006$), raised MAP (R=1.49; 95% CI=1.11, 1.82; $p=0.006$), and use of CNI (R=1.15; 95% CI=1.04, 1.28; $p=0.008$).

In the adjusted model, the only independent predictors of raised sE-selectin levels were lower adiponectin (R=0.96; 95% CI=0.92, 0.99; $p=0.004$), elevated endotoxin (R=1.04; 95% CI=1.03, 1.04; $p=0.007$), increased hsCRP (R=1.65; 95% CI=1.11, 2.45; $p=0.02$), higher WC (R=1.35; 95% CI=1.11, 1.65; $p=0.005$), raised MAP (R=1.35; 95% CI=1.11, 1.82; $p=0.006$), and male (R=1.07; 95% CI=1.02, 1.13; $p=0.01$).

As shown in **Figure 3b**, there is a negative association between adiponectin and sE-selectin levels, but this association is only prominent when adiponectin levels fall below 20 μ g/mL.

Figure 3c demonstrated a positive linear relationship between endotoxin and sE-selectin levels, with 1-unit increase in endotoxin level associated with 4% increase in sE-selectin levels. The positive linear relationship between hsCRP and sE-selectin levels is shown in **Figure 3d**, a 10-unit increase in hsCRP level is associated with 6.5% increase in sE-selectin level. In addition, the positive association between WC and sE-selectin is only evident when WC is greater than 100cm, shown in **Figure 3e**. Likewise, a positive association was observed between MAP and sE-selectin (**Figure 3f**), but this association only holds true with MAP higher than 100mmHg. Moreover, males were found to have 7% higher sE-selectin levels compared to female counterpart. Finally, borderline effects of advancing age ($R=1.22$; 95% CI=0.99, 1.49; $p=0.07$) and use of CNI ($R=1.09$; 95% CI=0.99, 1.20; $p=0.06$) were observed in the final multivariate regression model. However, such associations did not reach statistical significance. On average, a 10-year increase in age is associated with 2.2% increase in sE-selectin levels; and the use of CNI is associated with 9% higher sE-selectin levels compared with non-use of CNI. A 47% of the variation in sE-selectin was explained by the variables in the final multivariate model ($R^2=46\%$, **Table 4**).

Discussion

Although the adverse impact of inflammation on patient and graft outcomes in KTRs is uncontested, the drivers of this inflammatory response are incompletely understood, and therefore hinders adoption of a rational therapeutic approach. Similarly, the relationship between inflammation and vascular disease is subject to confounders by its underlying causes, which are, hitherto unexplored in detail in the field of kidney transplantation. This

study aimed to clarify these relationships, giving rise to plausible underlying mechanisms and therapeutic targets.

This study represents the first evidence in kidney transplantation showing that endotoxemia is a significant independent predictor of inflammation in KTRs, and extends the relationship seen in non-transplantation CKD¹⁶, haemodialysis¹⁶, and peritoneal dialysis³⁸ populations. Endotoxin, also known as lipopolysaccharide (LPS), is found in the outer cell membrane of the cell wall of Gram-negative bacteria that reside in the intestinal lumen as part of gut microbiota¹⁷. Upon release into the circulation, LPS stimulates the release of pro-inflammatory cytokines, resulting in the ‘syndrome’ of systemic inflammation. Of note, the association between endotoxin and hsCRP levels remains minimal up to approximately 2.5 EU/mL, thereafter an exponential increase in inflammation was seen with increasing endotoxemia. This is in line with the conventional believe that endotoxemia exists with endotoxin level rises higher than 2.5 EU/mL³⁹.

Furthermore, it is well recognised that LPS induces endothelial activation and dysfunction¹⁷. Indeed, endotoxin levels were positively correlated with the levels of circulating sE-selectin, the measure of systemic endothelial damage used in the current study. It is important to note that this effect of endotoxemia was independent of inflammation, supporting a direct pathogenic role of endotoxin on maladaptation of endothelial cells to fibroblast phenotype without invoking an immune response, a relatively novel mechanism of endothelial fibrosis in the absence of immune cells⁴⁰. Other independent predictors of raised sE-selectin in this study included the well-recognised cardiovascular risk factors such as increased waist circumference, male gender and raised blood pressure, with some evidence for an effect of older age and the use of calcineurin inhibitors. Of interest, an association between reduced

levels of adiponectin and sE-selectin was found, this potentially important relationship has previously been recognised in animal model and pre-clinical settings^{41,42}, it is now extended to kidney transplantation.

On the basis of the above discussion, it is important to identify modifiable causes of endotoxemia. The current study suggests some potential therapeutic manoeuvres. It shows for the first time in the field of kidney transplantation that endotoxemia is associated with increased intakes of dietary fructose and saturated fats, raised serum triglycerides, higher waist circumference, and reduced levels of 25-hydroxyvitamin D. Importantly, these represent readily modifiable targets amenable to dietary and lifestyle modification.

The dietary contribution to endotoxemia may be explained by findings extrapolated from pre-clinical settings and general population, which show that fructose and saturated fat ingestions are associated with intestinal bacterial dysbiosis⁴³⁻⁴⁵, this in turn modulate intestinal tight junction integrity, and subsequently increasing intestinal permeability, bacterial translocation and endotoxemia, with the downstream inflammatory consequences described above⁴⁶. It should be noted that increased endotoxin levels were observed with fructose ingestion greater than 75g/day, suggesting that only excessive dietary intake is associated with this effect. The relationship between increased saturated fat intake and endotoxemia was only evident in the univariate analysis, although the independent association between higher waist circumference and endotoxemia may represent a surrogate for saturated fat intake⁴⁷. The independent association between lower circulating vitamin D levels and endotoxemia also has an evidence base outside transplantation⁴⁸, and a defined mechanism⁴⁸.

The relationship between waist circumference and endotoxemia is less easily explicable. On one hand, diet-induced obesity and genetic obesity are associated with adverse changes in gut microbiota composition, impairing gut barrier function and hence promoting metabolic endotoxemia⁴⁹. On the other hand, it may even represent a situation of ‘reverse causality’ whereby LPS on binding to CD14 on adipocytes serves as a trigger for obesity⁵⁰. Further interventional studies are required to explore these relationships in detail, but the current work serves to generate hypotheses which are amenable to future testing. In addition, it is highly likely that studying the enteric microbiome, which is outside the scope of the current study, will provide insight into the basic science of our findings.

In addition to the above-mentioned relationships with endotoxemia, increased fructose intake, reduced vitamin D levels, and increased waist circumference displayed associations with inflammation which were independent of the relationship between endotoxemia and inflammation. The mechanisms by which excessive fructose intake results in systemic inflammation are recognised, but the current study represents the first to describe this relationship in kidney transplantation. Similarly, reduced vitamin D levels as drivers of inflammation has been described in varied clinical scenarios^{51,52}, but not detailed in kidney transplantation. In contrast, chronic systemic inflammation in obesity, originating from local immune responses in visceral adipose tissue⁵³ is already recognised in kidney transplantation¹², and further confirmed in the current study. Of interest, the reciprocal relationship between dietary fibre intakes and inflammation is a novel finding of this study, particularly when dietary fibre intake falls below 15g/day. This relationship is consistent with findings from previous studies in the general population⁵⁴⁻⁵⁶. Although the mechanism

is incompletely elucidated, it has been suggested that dietary fibre decreases lipid oxidation and downstream inflammation⁵⁵.

This study has limitations that should be acknowledged. It represents a single-centre observational experience, and needs interpretation in this context. There was little variation in the use of immunosuppressive medication, although this homogeneity perhaps helps with identification of biologically plausible predictors of endotoxemia, inflammation and endothelial dysfunction in this study. Indeed, the analysis demonstrates that the identified predictors are responsible for an impressively large proportion of the variation in outcome. Long-term longitudinal follow-up and more detailed understanding of the basic science behind these observations will be of benefit. More importantly, this study sets the scene for the implementation of achievable interventions designed to improve cardiovascular outcome in kidney transplantation.

Acknowledgement

Winnie Chan received a research grant from the British Renal Society, and was awarded a PhD research training fellowship from the West Midlands Strategic Healthy Authority. The research was carried out at the National Institute of Health Research (NIHR) / Wellcome Trust Clinical Research Facility Birmingham. The views expressed are those of the authors and not necessarily those of the National Health Service and the NIHR of the Department of Health. The authors would like to thank the staff in the Renal Outpatients Department and the Wellcome Trust Clinical Research Facility for supporting this study. Also, special thanks to Golaleh McGinnell, Theresa Brady and Helen Houston for leading the nursing support of this research.

Disclosures

The authors declare no conflict of interest.

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