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

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Winnie Chan, Jos A Bosch, Anna C Phillips, Philip G McTernan, and Richard Borrows analysed the data and performed the statistical analysis.

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Short Title

Endotoxemia in Kidney Transplantation

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Abstract

Objective: Cardiovascular disease is the leading cause of death in kidney transplant recipients (KTRs), yet incompletely accountable by traditional risk factors. Inflammation is an unconventional cardiovascular risk factor, with gut-derived endotoxemia potentially driving inflammation and endothelial disease. Comparable data are lacking in kidney transplantation. This study investigated the associations of endotoxemia with inflammation, endothelial activation, and 5-year cardiovascular events in KTRs. Determinants of endotoxemia were also explored.

Design, Setting and Subjects: This is a single-centre cross-sectional study with prospective follow-up from a prevalent cohort of 128 KTRs.

Main Outcome Measures: Demographic, nutritional and clinical predictors of inflammation (high-sensitivity C-reactive protein; hsCRP), endothelial activation (sE-selectin) and endotoxemia (endotoxin) were assessed. Follow-up data on 5-year cardiovascular event rates were collected.

Results: Endotoxemia (p=0.03), reduced 25-hydroxyvitamin D (p=0.04), high fructose intake (p<0.001), decreased fibre intake (p<0.001), and abdominal obesity (p=0.002) were independently associated with elevated hsCRP. In turn, endotoxemia (p=0.007) and increasing hsCRP (p=0.02) were both independently associated with raised sE-selectin. Furthermore, endotoxemia predicted increased cardiovascular event rate (p=0.02), independent of hsCRP and a global measure of cardiovascular risk estimated by a validated algorithm of 7-year risk for major adverse cardiac events in kidney transplantation. Determinants of endotoxemia included reduced 25-hydroxyvitamin D (p<0.001),

hypertriglyceridemia (p<0.001), increased fructose intake (p=0.01), and abdominal obesity (p=0.01).

Conclusions: Endotoxemia in KTRs contributes to inflammation, endothelial activation, and increased cardiovascular events. This study highlights the clinical relevance of endotoxemia in KTRs, suggesting future interventional targets.

Introduction

Cardiovascular disease (CVD) is a leading cause of death and a major driver of graft loss in kidney transplant recipients (KTRs) [1]. Conventional cardiovascular risk factors incompletely explain the increased incidence of cardiovascular events in KTRs [2], and several studies have highlighted the potential contributions of non-traditional exposures [2-4]. Inflammation and immune reactivity are believed to provoke atherogenesis in the general population [5], whilst inflammation correlates with endothelial dysfunction and accelerated atherosclerosis in general [6] and chronic kidney disease (CKD) [6-9] populations. Although less studied in KTRs, recent evidence confirms that inflammation is 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, factors contributing to inflammation in KTRs remain under-investigated. It is also unclear whether such adverse outcomes are due to inflammation *per se*, or whether it reflects the underlying drivers of inflammatory processes. Abdominal obesity and smoking were identified in one study as important modifiable determinants of inflammation among KTRs [12], but aside from this, data in kidney transplantation remains limited. Yet studies in general and other diseased populations have identified important factors contributing to inflammation and endothelial dysfunction, with the role of systemic endotoxemia in this context having received attention recently.

Endotoxemia is characterised by the presence of endotoxins in the blood. Endotoxin, also known as Lipid A, is a core component of lipopolysaccharide (LPS) found in the outer cell

membrane of the cell wall of Gram-negative bacteria that reside in the intestinal lumen as part of gut microbiota [16]. Upon release into the circulation, LPS stimulates the release of pro-inflammatory cytokines and expression of surface adhesion molecules such as CD14 on inflammatory cells, resulting in the 'syndrome' of systemic inflammation [17]. *In vitro*, endotoxin induces endothelial damage and activation [16]. Clinical data shows that systemic endotoxemia is associated with cardiovascular disease in the general population [18], atherosclerosis in patients undergoing peritoneal dialysis [19], and increased mortality risk in haemodialysis patients [20,21]. The association between CD14 and mortality in CKD [22] and haemodialysis cohorts [23,24] further lend support to these observations. However, the role of endotoxemia driving inflammation and endothelial damage among KTRs remains unexplored and warrants further investigation.

This study therefore set out to investigate the role of endotoxemia on inflammation and endothelial activation in clinically stable KTRs, alongside traditional cardiovascular risk factors and contemporary risk factors such as hypovitaminosis D [25-28], hyperuricemia [29-31], hypoadiponectinemia [32-34], and high dietary intake of fructose [35-37]. The association between endotoxemia and 5-year cardiovascular events was also assessed. Finally, determinants of endotoxemia were explored, in an attempt to shed new light on plausible interventional targets to improve cardiovascular outcome in kidney transplantation.

Methods

Participants and Study Design

This is a cross-sectional study with prospective follow-up from a prevalent cohort of KTRs beyond 1-year post-transplantation with stable graft function (<10% increase in serum creatinine over the preceding 6 months). Patient recruitment occurred 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 (i.e. implanted or external electronic devices, metallic implants, amputations, pregnancy, and lactation). This study was approved by the local research ethics committee and was conducted in accordance with the Declaration of Helsinki.

Data Collection

Demographic and Clinical Parameters

Age, gender, ethnicity, and time post-transplantation were collected from patients' medical records. Alcohol intake (units per week) and smoking status were collected by questionnaire. Smoking status were classified into non-smoker, ex-smoker and current smoker, with ex-smokers defined as individuals who had ever smoked but did not smoke at the time of research visit, regardless of their age and length of time since quitting [38]. In addition, the following clinical parameters were retrieved from patients' medical records: 1) presence of coronary heart disease; 2) presence of diabetes, either pre-transplantation (pre-DM), or new

onset diabetes after transplantation (NODAT); 3) previous acute rejection episodes; 4) dialysis vintage; 5) number of kidney transplants; 6) pre-emptive transplantation; 7) use of statin; and 8) immunosuppressive medication usage, either prednisolone, calcineurin inhibitor (CNI), or adjunctive antiproliferative agent. Comorbidity was assessed by Index of Coexistent Disease (ICED) using the algorithm described by the Haemodialysis Study [39]. Cardiovascular risk was estimated by 7-year risk for major adverse cardiac events (MACE) in kidney transplantation using a validated algorithm derived by Soveri et al [40,41]. The required data for calculations of ICED and 7-year risk for MACE were extracted from patients' medical records.

Systolic (SBP) and diastolic (DBP) blood pressure 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 [42].

Cardiovascular Event Data

Following data collection at initial study evaluation, time to cardiovascular event of acute origin (i.e. coronary artery disease including acute coronary syndrome or myocardial infarction; and cerebrovascular disease including transient ischaemic attack or cerebrovascular accident) was extracted from patients' medical records. Such events were

classified according to clinical presentation at the time of occurrence. Follow-up period was up to 5 years post initial evaluation.

Laboratory Parameters

Blood samples were taken in the morning following an overnight fast for measurements of creatinine, estimated glomerular filtration rate (eGFR; derived using four-variable modifications of diet in renal disease equation), urate, 25-hydroxyvitamn D, and full lipid profile including total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides. Analyses were undertaken in the 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, the latter chosen as the most specific circulating marker for endothelial activation [43], 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) [44]; 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), which has been widely validated as detailed previously [45,46]. The intra- and inter- assay coefficients of variation were 3.9% and 9.6% respectively [47].

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/m^2) was calculated as weight (kg) divided by height squared (m^2). 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 Organisation [48].

In addition, a well-validated multi-frequency bio-impedance based body composition monitor [49] (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/m^2), fat tissue index (FTI, kg/m^2), 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, a widely accepted dietary assessment tool deemed to be valid, reliable and accurate in estimating typical and habitual nutrient intake [50,51]. 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 23 (Chicago, IL). Results were presented as mean ± standard deviation (SD) for normally distributed data or median

(interquartile range, IQR) for non-normally distributed data. Independent-sample t-test was used to compare continuous data between groups.

Linear regression analysis was used to determine the associations between predictor variables and the continuously distributed outcome variables. Regression diagnostics were performed. The continuously distributed outcome variables (hsCRP, sE-selectin and endotoxin levels) demonstrated positively skewed non-Gaussian distributions, logarithmic transformations were performed prior to regression analyses. Time to cardiovascular event data was analysed by Cox regression, with Kaplan-Meier survival estimates for the relationship between endotoxemia and time to cardiovascular event evaluated by log-rank statistics. The ranges of endotoxin concentrations presented in the Kaplan Meier survival curve were tertiles determined following a post-hoc 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 ≤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.

Due to logarithmic transformation of the outcome variable, exponential of the beta coefficient has been applied, and hence beta coefficient and its associated 95% confidence interval (CI) have been transformed and reported in the form of "Ratio". For the categorical variable, the

ratio of the outcome in each category was relative to the outcome of the baseline category. For the continuous variable, unless otherwise stated, the ratio was given for one-unit increase in the explanatory variable.

Graphical linear and non-linear associations shown in **Figure 1**, **Figures 2a-2b**, and **Supplementary Material** were determined by GraphPad Prism version 6 (GraphPad Software, La Jolla California, USA) using either linear regression, exponential model, or second order polynomial (quadratic) lines of best fit.

Results

Patient Characteristics

Of 138 patients approached, 10 did not participate due to work commitment (93% consent rate). Mean age was 49 ± 15 years, 56% were male, 78% were Caucasian, 29% and 9% were ex-smoker and current smoker respectively, 20% had either pre-DM or NODAT, mean eGFR was 48 ± 18 mL/min, and mean SBP and DBP were 140 ± 19 and 80 ± 10 mmHg respectively. Immunosuppressive medication usage among KTRs were: 78% on prednisolone; 91% on calcineurin inhibitor; and 87% on adjunctive antiproliferative agent. Statin usage was 55%. Median endotoxin level was 1.95 (1.49 – 2.38) EU/mL. Median hsCRP level was 2.47 (1.00 – 4.89) mg/L. Median sE-selectin level was 34.2 (24.1 – 44.8) ng/mL. Full details of patient characteristics of the studied population are shown in **Table 1**.

Determinants of Inflammation (hsCRP) in KTRs

Table 2 indicates the predictors of inflammation in KTRs. Increased endotoxin concentration was an independent predictor of hsCRP in the final multivariate regression model (p=0.03). **Figure 1**, shows the relationship between hsCRP and serum endotoxin concentration, demonstrating the marked increase in hsCRP with endotoxin level above 2.5 EU/mL. Other independent predictors of hsCRP included decreased 25-hydroxyvitamn D levels (p=0.04; **Supplementary Material, Figure S1a**), high fructose intake (p<0.001; **Supplementary Material, Figure S1b**), decreased dietary fibre intake (p<0.001; **Supplementary Material, Figure S1c**), and increasing WC (p=0.002; **Supplementary Material, Figure S1d**).

In addition, univariate associations between the following predictor variables and hsCRP were seen, but did not hold in the adjusted model: increasing urate concentrations (p=0.007), increased LDL (p=0.01), higher saturated fat intake (p=0.03) and increased FTI (p<0.001).

Of note, a substantial proportion of the variation in hsCRP was explained by the variables contained within the final model (R^2=67%).

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

Predictors of sE-selectin levels are detailed in **Table 3**. Both increased endotoxin and raised hsCRP levels were independent predictors of elevated sE-selectin levels in the multivariate regression analysis (p=0.007, **Figure 2a**; and p=0.02, **Figure 2b** respectively). Other significant independent predictors included lower adiponectin concentrations (p=0.004; **Supplementary Material, Figure S2a**), higher WC (p=0.005; **Supplementary Material, Figure S2b**), raised MAP (p=0.006; **Supplementary Material, Figure S2c**), and male gender (p=0.01). In addition, some evidence for an effect of increasing age (p=0.07) and use of CNI (p=0.06) were seen in the final model.

Univariate associations with sE-selectin were also seen with decreased HDL (p=0.02), increased triglycerides (p=0.001), higher fructose intake (p=0.04), and lower LTI (p=0.004), However, these associations did not persist in the adjusted model.

The variables within the final regression model explained 47% of the variation in sE-selectin levels (R^2=46%).

Determinants of Endotoxin Levels in KTRs

Table 4 shows the predictors of endotoxemia. Independent predictors of increased endotoxin concentrations in the final multivariate regression model were as follows: decreased 25-hydroxyvitamn D levels (p<0.001; **Supplementary Material, Figure S3a**), higher

triglycerides (p<0.001; **Supplementary Material, Figure S3b**), higher fructose intake (p=0.01; **Supplementary Material, Figure S3c**), and increasing WC (p=0.01; **Supplementary Material, Figure S3d**). Borderline effects of reduced LTI (p=0.07), pre-DM and NODAT (p=0.08) were seen in the final model.

Univariate associations between increased serum endotoxin concentration and decreased HDL (p=0.006), increased LDL (p<0.001), raised total cholesterol (p=0.004), higher intake of saturated fat (p=0.04), and increased FTI (p<0.02) were also seen, but did not persist in the adjusted model.

The predictor variables within the final regression model explained 46% of the variation in endotoxin levels (R^2=46%).

Association between Endotoxemia and Cardiovascular Events

Following initial evaluation, all patients underwent prospective follow-up at the same centre for a median duration of 51 (IQR 48-58) months. Six patients suffered an acute cardiovascular event during the follow-up period (acute coronary syndrome=3; myocardial infarct=1; ischaemic cerebrovascular accident=1; new onset lower limb claudication=1). Endotoxin levels were significantly higher in these patients compared to those without cardiovascular event during the follow-up period (2.85±0.26 EU/mL versus 1.95±0.06 EU/mL respectively; p<0.001). Univariate Cox regression analysis revealed a significant

association between endotoxin level and time to cardiovascular event (Hazard Ratio, HR, per EU/mL=2.34, 95% CI=1.23, 4.44, p=0.01). This analysis was then adjusted for hsCRP and 7-year risk of MACE, the latter as a validated composite surrogate for global cardiovascular risk [40,41]. Following adjustment, endotoxin level remained a significant independent predictor of cardiovascular event (HR per EU/mL=2.22, 95% CI=1.11, 4.43, p=0.02); 7-year risk of MACE also showed a significant independent relationship with time to cardiovascular event (HR per %=1.04, 95% CI=1.01, 1.08, p=0.04). No evidence of an association with hsCRP was seen in the adjusted analysis (HR per mg/L=1.01, 95% CI=0.94, 1.08, p=0.87). Kaplan-Meier survival estimates for the relationship between endotoxemia (by tertile) and time to cardiovascular event are shown in **Figure 3**.

Discussion

Although the adverse impact of inflammation on patient and graft outcomes in KTRs is well-recognised, the drivers of such inflammatory responses are incompletely understood, therefore impeding the development of therapeutic strategies. Similarly, the relationship between inflammation and vascular disease is subject to confounders by its underlying causes, many of which remain unexplored in kidney transplantation. This study aimed to clarify these relationships by exploring the plausible underlying mechanisms, pointing towards potential therapeutic targets.

This study represents the first evidence in kidney transplantation showing that endotoxemia may be a significant independent predictor of inflammation in KTRs. This finding extends

the relationship observed in non-transplant CKD [20], haemodialysis [20], and peritoneal dialysis [19] populations. In addition, endotoxemia was identified as a possible independent risk factor for endothelial activation in this study, a novel finding in kidney transplantation. Furthermore, this study provides preliminary insight in kidney transplantation that raised endotoxin levels may be associated with increased cardiovascular events, an effect independent of inflammation or global cardiovascular risk. However, it is important to note that the cardiovascular event rates were low in this study, therefore this finding needs to be validated in larger cohort with longer follow-up duration. Nevertheless, such observations are noteworthy in light of the increased cardiovascular risk seen in KTRs, and yet incompletely explained by traditional cardiovascular risk factors. Of clinical relevance, these results provide insights for potential therapeutic options. In addition, it suggests for the first time in kidney transplantation that increased dietary intakes of fructose and saturated fats, higher waist circumference, and reduced levels of 25-hydroxyvitamin D are possible drivers of endotoxemia in this context. The findings from this study support and extend data from the haemodialysis literature, showing potential associations between endotoxemia and high BMI [21] as well as elevated triglyceride levels [23]. Collectively, these findings suggest novel targets modifiable by dietary and lifestyle interventions.

In vitro study demonstrates the phenomenon of endotoxin-induced endothelial activation and dysfunction [16]. This is in line with the current study showing that endotoxin levels were positively correlated with levels of circulating sE-selectin, the most specific circulating marker of endothelial activation [43], with the latter being independently associated with incident coronary artery disease and the presence of carotid atheroma in general populations [52]. The current study also extends this clinical data to a transplant cohort, whereby an

association between endotoxemia and increased cardiovascular events of acute origin was observed. Importantly, this association was independent of a validated composite marker of global cardiovascular risk, as previously derived by Soveri et al [40,41]. Pertinently, this composite marker was also independently associated with cardiovascular events, supporting the face validity of our findings. Such effect of endotoxemia observed in this study was also independent of inflammation, although a systemic inflammatory response, assessed by hsCRP, was also in itself associated with elevated sE-selectin levels. The 'inflammation-independent' effect of endotoxemia upon endothelial activation found in this study resonates with a recently described mechanism, whereby endotoxin induces endothelial cells to display fibroblast-like phenotypes resulting in endothelial fibrosis, but in the absence of an invoked immune response [53]. Nevertheless, it remains a possibility that other inflammation-related mechanisms may also lead to endothelial activation and injury. In fact, an inflammatory response was observed with increasing endotoxin concentration in the current study. Specifically, increased hsCRP level was most evident beyond endotoxin concentration of 2.50 EU/mL. This finding is in keeping with the prior suggestion that clinically relevant endotoxemia exists at levels greater than 2.50 EU/mL [54]. Additionally, previous studies showed that endotoxin levels among healthy individuals, non-dialysis CKD, haemodialysis, and peritoneal dialysis patients were 0.01 EU/mL [19], 0.03 – 0.11 EU/mL [19,20], 0.64 - 0.69 EU/mL [55], and 0.44 – 0.56 EU/mL [19,20] respectively. The median endotoxin concentration level in the current cohort of KTRs was 1.95 EU/mL, substantially higher than those observed in healthy, non-dialysis and dialysis-dependent CKD populations. Taken together, this study supports the concept that endotoxemia poses an increased cardiovascular risk in KTRs, extending the evidence-base from general and dialysis populations, and suggests the potential adverse impact of endotoxemia on cardiovascular outcomes. Therefore, strategies aiming to reduce endotoxin levels among KTRs should be considered.

Hence, it can be hypothesised that modifying endotoxemia may represent a useful strategy in the management of inflammation and cardiovascular risk in kidney transplantation. The findings from the earlier part of this study raise intriguing questions regarding possible interventions to combat endotoxemia. The results presented in the latter part of this study proposed a number of such therapeutic options. Firstly, lifestyle modification may be appropriate. In particular, the positive associations between endotoxemia and saturated fat intakes as well as excessive fructose intakes may provoke intestinal bacterial dysbiosis [56-58], which in turn modulates intestinal tight junction integrity, increasing intestinal permeability and bacterial translocation, ultimately causing endotoxemia [59]. Therefore, minimising dietary intakes of saturated fats and fructose may be appropriate. At present, recommendation on fructose intake in KTRs remains unavailable; results from the current study showed minimal effects of fructose intake on increasing endotoxemia and inflammation until it reaches approximately 75g per day. These findings are in line with the general consensus that only excessive fructose consumption of ≥50 – 100g/day is associated with adverse health implications [60]. Secondly, the relationship between endotoxemia and increasing WC observed in the present study further supports the potential role of lifestyle intervention. This relationship may be explained by the effect of diet-induced obesity or genetic obesity [61], whereby adverse changes in composition of gut microbiota were found in obese conditions, impairing gut barrier function, and hence promoting 'metabolic endotoxemia' [61]. Indeed, the mean WC measurements in the current cohort of KTRs were 105cm for males and 89cm for females, both of which were higher than the World Health Organisation cut-off points for risk of metabolic complications (>102cm for males; and >88cm for females) [62]. Therefore, strategies for targeting abdominal obesity among KTRs should be in place. Finally, the independent association between endotoxemia and lower circulating levels of 25-

hydroxyvitamin D suggests that dietary supplementation and sunlight exposure may be appropriate in this setting. The inverse relationship between endotoxin and 25-hydroxyvitamin D levels is supported by data existing outside the field of kidney transplantation, whereby vitamin D deficiency leads to defective gut mucosal integrity and immunity, allowing entry of endotoxin into the systemic circulation [63]. In addition, systemic clearance of LPS is impaired due to blunting of innate immunity by low circulating vitamin D level, resulting in endotoxemia [63]. Accordingly, findings from the current study serve to generate hypotheses amendable to future testing, with interventional studies required to explore these relationships in detail. It is likely that the evaluation of enteric microbiome in kidney transplantation, which was outside the scope of the current study, may provide insight into the basic science of these findings.

Of note, increased fructose intake, reduced 25-hydroxyvitamin D levels, and increased WC also displayed associations with inflammation independent of the relationship between endotoxemia and inflammation. The mechanisms by which excessive fructose intake results in systemic inflammation are recognised [36], but the current study represents the first to describe this possible relationship in kidney transplantation. Similarly, low vitamin D levels as possible drivers of inflammation has been described in other clinical settings [64,65], but not detailed in kidney transplantation. The chronic systemic inflammation of obesity, originating from local immune responses in visceral adipose tissue [66], is recognised in kidney transplantation [12], and further confirmed in the current study. Of interest, the reciprocal relationship between dietary fibre intake and inflammation in KTRs is a novel finding of this study; it was most evident when dietary fibre intake fell below 15g/day. This relationship is consistent with findings from the general population [67-69]. Although the mechanism is

incompletely elucidated, it has been suggested that dietary fibre possibly decreases lipid oxidation and downstream inflammation [67].

Finally, the association between reduced levels of adiponectin and endothelial activation is another novel and biologically plausible finding of this study. It extends the recognised protective effects of adiponectin on endothelium from animal models and pre-clinical settings [70,71] to kidney transplantation. Other independent predictors of endothelial activation in this study, and perhaps testament to the face validity of the current findings, included the well-recognised cardiovascular risk factors such as increased WC, male gender and raised blood pressure, with some evidence for an effect of aging and the use of CNI. Of note, sE-selectin is considered the most specific circulating marker of endothelial health. It reflects cotemporaneous endothelial activation rather than established structural vascular disease [43,72]. In the context of kidney transplantation, structural vascular disease reflects a multiplicity of prior comorbidities including those accumulated during the period of CKD and dialysis, as well as ongoing endothelial injury [73]. Since the latter represented the focus of the current study, a specific endothelial activation marker (i.e. sE-selectin) was chosen for the analysis.

Interestingly, the findings from this study showed that the use of statins did not have an impact on inflammation in KTRs. The lack of anti-inflammatory effect may be accountable by the lack of lipid-lowering effect in this cohort of KTRs [74], as implicated by the mean levels of total- and LDL- cholesterol of 5.0 mmol/L and 3.1 mmol/L respectively, despite 55% of the patient population were prescribed statin albeit at low doses. In addition, smoking is known to promote inflammation and endothelial dysfunction [75]. It is somewhat surprising that tobacco use did not associate with markers of inflammation and endothelial

activation in the current cohort of KTRs. Nevertheless, based on statistical point estimates, current smoker displayed positive associations with hsCRP, sE-selectin and endotoxin levels whereas ex-smoker revealed negative associations with hsCRP, sE-selectin and endotoxin levels. The lack of observed statistical significance may be attributed to insufficient sample size with only 9% and 29% of smokers and ex-smokers respectively. Also, such a discrepancy may be related to classification bias since ex-smokers in this study encompassed both distant and recent ex-smokers irrespective of their length of time since quitting. Results from the National Health and Nutritional Examination Survey (NHANES III) showed that inflammation subsides only after 5 years of smoking cessation [76]. Therefore, the definition of "ex-smoker" employed in this study may not truly reflect the impact of tobacco exposure on inflammation and cardiovascular risk. Previous studies investigating the adverse impact of smoking in CKD and kidney transplant populations sub-classified "ex-smokers" into "distant ex-smokers" (quitted smoking >1 or 5 years) or recent ex-smokers (quitted smoking <1 or 5 years) [77-80]. Future studies aiming to evaluate the effects of smoking history on markers of inflammation and cardiovascular risk in KTRs should better delineate the "ex-smoker" category taking into account the duration of smoking cessation.

This study has limitations that should be acknowledged. It represents a single-centre observational experiment characterised by a small sample size and low cardiovascular event rates. Therefore, further validations are needed in larger cohorts. In addition, the use of 3-day food diary may be influenced by day-to-day and seasonal variations. The use of food frequency questionnaire should be included in future studies aiming to provide knowledge on habitual dietary intake over a longer period, enabling a more accurate assessment between the correlations of dietary intakes with inflammation, endothelial activation and endotoxemia.

Also, the studied cohort was predominately Caucasian, and hence the findings from this study may not be generalizable to other ethnic groups. Furthermore, there is little variation in the use of immunosuppression, but such an effect helps to enhance the homogeneity of the study. Indeed, the R^2 values generated from the multivariate statistical models demonstrate that the identified predictors are responsible for large proportions of the variation in outcome variables. Soluble and cell surface expressed CD14 levels, both of which are influenced by endotoxemia [81], were not examined in the current study, therefore previous data [17,22-24] describing their importance as exposures cannot be confirmed or refuted. The cross-sectional nature of this study is unable to establish causality between predictor and outcome variables; long-term longitudinal follow-up and a detailed understanding of the basic science behind the observations found in this study is crucial.

In summary, this study demonstrates potential targets for intervention, and sets the scene for future interventional research and therapeutic strategies in KTRs. In particular, targeting endotoxemia may serve as a potent upstream intervention for the management of cardiovascular risk in these patients, potentially improving medium- and long- term clinical outcomes of kidney transplantation.

Practical Application

This study represents the first evidence in kidney transplantation showing that endotoxemia may be a significant independent predictor of inflammation and endothelial activation. It provides preliminary insight into the association between endotoxemia and cardiovascular outcome in kidney transplantation. Our findings highlight the potential relevance of

endotoxemia and other unconventional cardiovascular risk factors in kidney transplantation, setting the scene for future interventional research and therapeutic strategies.

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Table Legends

Table 1	Population Characteristics
Table 2	Predictors of Inflammation (hsCRP) in Kidney Transplant Recipients
Table 3	Predictors of Endothelial Activation (sE-selectin) in Kidney Transplant Recipients
Table 4	Predictors of Endotoxemia (Endotoxin) in Kidney Transplant Recipients

Figure Legends

Figure 1	Association between hsCRP and Endotoxin Levels in Kidney Transplant Recipients
Figure 2a	Association between sE-selectin and Endotoxin Levels in Kidney Transplant Recipients
Figure 2b	Association between sE-selectin and hsCRP Levels in Kidney Transplant Recipients
Figure 3	Kaplan-Meier Estimates of Time-to-Cardiovascular Event in Kidney Transplant Recipients with Different Levels of Endotoxemia by Tertile

Supplementary Material, Figure Legends

Supplementary Material, Figure S1a	Association between hsCRP and 25-Hydroxylvitamin D Levels in Kidney Transplant Recipients
Supplementary Material, Figure S1b	Association between hsCRP Levels and Fructose Intake in Kidney Transplant Recipients
Supplementary Material, Figure S1c	Association between hsCRP Levels and Dietary Fibre Intake in Kidney Transplant Recipients
Supplementary Material, Figure S1d	Association between hsCRP Levels and Waist Circumference in Kidney Transplant Recipients
Supplementary Material, Figure S2a	Association between sE-selectin and Adiponectin Levels in Kidney Transplant Recipients
Supplementary Material, Figure S2b	Association between sE-selectin Levels and Waist Circumference in Kidney Transplant Recipients
Supplementary Material, Figure S2c	Association between sE-selectin Levels and Mean Arterial Pressure (MAP) in Kidney Transplant Recipients
Supplementary Material, Figure S3a	Association between Endotoxin and 25-Hydroxylvitamin D Levels in Kidney Transplant Recipients
Supplementary Material, Figure S3b	Association between Endotoxin and Triglyceride Levels in Kidney Transplant Recipients
Supplementary Material, Figure S3c	Association between Endotoxin Levels and Fructose Intake in Kidney Transplant Recipients
Supplementary Material, Figure S3d	Association between Endotoxin Levels and Waist Circumference in Kidney Transplant Recipients

Table 1. Population Characteristics

Demographic and Clinical Parameters	
Sample size (n)	128
†Mean age (years)	49 ± 15
Gender (%)	Male = 56 Female = 44
*Ethnicity (%)	Caucasian = 78 Asian = 15 Afro-Caribbean = 5 Others = 2
‡Median time post-transplantation (years)	4 (2-11)
Pre-emptive transplantation (%)	22
Number of kidney transplants (%)	One = 92 Two = 6 Three = 2
Dialysis vintage (days)	843 (414-1801)
Smoking status (%)	Non-smoker = 62 Current smoker = 9 Ex-smoker = 29
‡Median alcohol intake (units/week)	1 (1-3)
‡Median ICED score	2 (2-2)
Presence of coronary heart disease	23%
‡Median 7-year risk of MACE (%)	11 (6-19)
5-year cardiovascular event rate [acute cardiovascular event] (number of patients and %)	Number of patients = 6 % = 5
‡Median follow-up duration for cardiovascular event (months)	51 (48-58)
Presence of diabetes (%)	Pre-DM = 10 NODAT = 15 Non-diabetic = 75
Previous acute rejection episodes (%)	Yes = 9 No = 91
Immunosuppressive medication usage	
Prednisolone (%)	78
Calcineurin inhibitor (%)	91
Adjunctive antiproliferative agent (%)	87
Dosage of immunosuppressive medications	
‡Median dose of Prednisolone (mg/day)	5 (5-5)
‡Median dose of Tacrolimus (mg/day)	4.0 (2.5-7.4)
‡Median dose of Cyclosporin (mg/day)	150 (150-200)
†Mean dose of Mycophenolate Mofetil (mg/day)	1040 ± 402
†Mean dose of Azathioprine (mg/day)	82 ± 36
Lipid lowering medication usage	
Overall statin usage (%)	55
Simvastatin (%)	5
Atorvastatin (%)	7
Fluvastatin (%)	41
Pravastatin (%)	2
Dosage of lipid lowering medications	
‡Median dose of Simvastatin (mg/day)	30 (20-40)
‡Median dose of Atorvastatin (mg/day)	30 (10-40)
‡Median dose of Fluvastatin (mg/day)	40 (20-40)
‡Median dose of Pravastatin (mg/day)	20 (10-20)
Blood pressure	
†Mean SBP (mmHg)	140 ± 19
†Mean DBP (mmHg)	80 ± 11
†Mean MAP (mmHg)	101 ± 11
Laboratory Parameters	
†Mean urate (μmol/L)	420 ± 100
‡Median 25-hydroxyvitamin D (nmol/L)	42 (20-64)
†Mean eGFR (mL/min)	45 ± 18
‡Median creatinine (μmol/L)	132 (107-169)
Lipid profile	
†Mean total cholesterol (mmol/L)	5.0 ± 1.1
‡Median HDL (mmol/L)	1.5 (1.2-1.9)
†Mean LDL (mmol/L)	3.1 ± 0.9
‡Median triglycerides (mmol/L)	1.5 (0.9-2.2)
‡Median ACR (mg/mmol)	4.35 (1.63-14.7)
‡Median hsCRP (mg/L)	2.47 (1.00-4.89)
‡Median adiponectin (μg/mL)	10.25 (6.24-13.82)
‡Median sE-selectin (ng/mL)	34.2 (24.1-44.8)
‡Median endotoxin (EU/mL)	1.95 (1.49-2.38)
Anthropometric Measurements and Body Composition Parameters	
†Mean BMI (kg/m^2)	All: 28.1 ± 5.7 Male: 29.1 ± 5.6 Female: 26.8 ± 5.8
†Mean WC (cm)	All: 98 ± 17 Male: 105 ± 16 Female: 89 ± 14
Bio-impedance measurements	
†Mean LTI (kg/m^2)	All: 13.5 ± 3.8
†Mean FTI (kg/m^2)	All: 13.8 ± 6.3
†Mean volume expansion (%)	All: 2.8 ± 7.8
Dietary Intake Parameters	
‡Median fructose intake (g)	16.9 (9.0-26.7)
‡Median dietary fibre intake (g)	15.8 (12.0-20.9)

For the purpose of statistical analysis, the ethnicity of patients classified as "Afro-Caribbean", "Asian" and "Others" was grouped as "Non-Caucasian", 78% "Caucasian" versus 22% "Non-Caucasian".
†Normally distributed data, results expressed as mean ± standard deviation (SD).
‡Non-normally distributed data, results expressed as median (interquartile range, IQR).
Abbreviations: **ACR**=Albumin-to-Creatinine Ratio; **BMI**=Body Mass Index; **DBP**=Diastolic Blood Pressure; **eGFR**=estimated Glomerular Filtration Rate; **FTI**=Fat Tissue Index; **HDL**=High-Density Lipoprotein; **hsCRP**=high-sensitivity C-Reactive Protein; **ICED**=Index of Coexistent Disease; **LDL**=Low-Density Lipoprotein; **LTI**=Lean Tissue Index; **MACE**=Major Adverse Cardiac Events; **MAP**=Mean Arterial Pressure; **NODAT**=New Onset Diabetes After Transplantation; **Pre-DM**=Presence of Diabetes Mellitus pre-transplantation; **SBP**=Systolic Blood Pressure; **sE-selectin**=soluble E-Selectin; **WC**=Waist Circumference.

Table 2. Predictors of Inflammation (hsCRP) in Kidney Transplant Recipients

	Univariate Analysis		Multivariate Analysis[§]	
	Ratio[δ] (95% CI[∞])	*p*-value	Ratio[δ] (95% CI[∞])	*p*-value
Laboratory Parameters				
**Adiponectin (μg/mL)	0.81 (0.22, 2.72)	0.72		
Endotoxin (EU/mL)	1.20 (1.07, 1.34)	0.002	1.20 (1.08, 1.33)	0.03
**Vitamin D (nmol/L)	0.67 (0.49, 0.90)	0.004	0.82 (0.74, 1.00)	0.04
**Urate (μmol/L)	1.11 (1.00, 1.22)	0.007		
**eGFR (mL/min)	0.74 (0.50, 1.22)	0.28		
HDL (mmol/L)	0.87 (0.74, 1.02)	0.08		
LDL (mmol/L)	1.13 (1.03, 1.23)	0.01		
*Total Cholesterol (mmol/L)	1.78 (0.80, 3.93)	0.15		
*Triglycerides (mmol/L)	1.97 (0.90, 4.31)	0.09		
Anthropometric Measurements and Body Composition Parameters				
*WC (cm)	1.12 (1.06, 1.16)	<0.001	1.05 (1.02, 1.08)	0.002
*FTI (kg/m^2)	1.39 (1.23, 1.58)	<0.001		
*LTI (kg/m^2)	1.20 (0.96, 1.51)	0.11		
*Volume Expansion (%)	1.00 (0.90, 1.12)	0.95		
Dietary Intake Parameters				
*Fructose intake (g)	1.13 (1.12, 1.15)	<0.001	1.12 (1.09, 1.13)	<0.001
*Dietary fibre intake (g)	0.85 (0.70, 0.90)	<0.001	0.85 (0.79, 1.08)	<0.001
*Total fat intake (g)	0.82 (0.61, 1.11)	0.28		
*Saturated fat intake (g)	1.14 (1.10, 1.18)	0.03		
Demographic and Clinical Parameters				
**Age (years)	1.00 (0.55, 1.82)	0.95		
Gender				
Female	1.00	0.72		
Male	1.03 (0.87, 1.22)			
[†]**Ethnicity**				
Caucasian	1.00	0.39		
Non-Caucasian	0.95 (0.84, 1.07)			
**Time post transplantation (years)	1. 65 (0.50, 6.05)	0.38		
Pre-emptive transplantation				
No	1.00	0.21		
Yes	0.88			
Previous episodes of acute rejection				
No	1.00	0.32		
Yes	1.15 (0.87, 1.54)			
**Dialysis vintage (years)	1.05 (0.99, 1.07)	0.07		
ICED	1.13 (0.88, 1.44)	0.34		
Presence of diabetes				
Non-diabetic	1.00	0.12		
NODAT	1.11 (0.97, 1.26)			
Pre-DM	1.15 (0.94, 1.39)			
**MAP (mmHg)	1.11 (0.49, 2.23)	0.88		
Smoking status				
Non-smoker	1.00	0.26		
Ex-smoker	0.87 (0.72, 1.04)			
Current smoker	1.17 (0.87, 1.58)			
Ex-smoker / Current smoker	0.94 (0.79, 1.11)			
Alcohol intake (units/week)	0.98 (0.96, 1.01)	0.24		
Use of statin				
No	1.00	0.78		
Yes	0.98 (0.82, 1.16)			
Use of calcineurin inhibitor				
No	1.00	0.19		
Yes	0.83 (0. 62, 1.10)			
Use of adjunctive antiproliferative agents				
No				
Yes	1.00	0.24		
	1.08 (0.95, 1.24)			
Use of prednisolone				
No	1.00	0.41		
Yes	1.09 (0.89, 1.33)			
Adjusted R^2 from final model			67%	

[§]Results in the final multivariate regression model were presented.

[δ]Due to the logarithmic transformation of the outcome variable, exponential of the beta coefficient has been applied, and hence beta coefficient and its associated 95% CI have been transformed and reported in the form of "Ratio". For the categorical variable, the ratio of the outcome in each category was relative to the outcome in the baseline category. For the continuous variable, unless otherwise stated, the ratio was given for one-unit increase in the explanatory variable.

[∞]CI = Confidence Interval.

[†]For the purpose of statistical analysis, the ethnicity of patients classified as "Afro-Caribbean", "Asian" and "Others" was grouped as "Non-Caucasian", 78% "Caucasian" versus 22% "Non-Caucasian".

Abbreviations: **eGFR**=estimated Glomerular Filtration Rate; **FTI**=Fat Tissue Index; **HDL**=High-Density Lipoprotein; **hsCRP**=high-sensitivity C-Reactive Protein; **ICED**=Index of Coexistent Disease; **LDL**=Low-Density Lipoprotein; **LTI**=Lean Tissue Index; **MAP**=Mean Arterial Pressure; **NODAT**=New Onset Diabetes After Transplantation; **Pre-DM**=Presence of Diabetes Mellitus pre-transplantation; **WC**=Waist Circumference.

(*) Coefficients reported for a 10-unit increase in explanatory variable.

(**) Coefficients reported for a 100-unit increase in explanatory variable.

Table 3. Predictors of Endothelial Activation (sE-selectin) in Kidney Transplant Recipients

	Univariate Analysis		Multivariate Analysis[§]	
	Ratio[δ] (95% CI[∝])	*p*-value	Ratio[δ] (95% CI[∝])	*p*-value
Laboratory Parameters				
*Adiponectin (μg/mL)	0.94 (0.89, 0.98)	0.007	0.96 (0.92, 0.99)	0.004
Endotoxin (EU/mL)	1.09 (1.05, 1.14)	<0.001	1.04 (1.03, 1.05)	0.007
**Vitamin D (nmol/L)	0.99 (0.90, 1.11)	0.85		
**Urate (μmol/L)	1.00 (0.97, 1.04)	0.46		
**hsCRP (mg/L)	1.49 (1.00, 2.22)	0.04	1.65 (1.11, 2.45)	0.02
**eGFR (mL/min)	0.90 (0.82, 1.11)	0.51		
HDL (mmol/L)	0.93 (0.88, 0.99)	0.02		
LDL (mmol/L)	1.03 (0.99, 1.07)	0.06		
Total Cholesterol (mmol/L)	1.01 (0.98, 1.04)	0.37		
Triglycerides (mmol/L)	1.05 (1.02, 1.08)	0.001		
Anthropometric Measurements and Body Composition Parameters				
**WC (cm)	1.35 (1.11, 1.64)	0.003	1.35 (1.11, 1.65)	0.005
**FTI (kg/m^2)	1.22 (0.74, 2.01)	0.401		
LTI (kg/m^2)	0.98 (0.97, 0.99)	0.004		
**Volume Expansion (%)	0.82 (0.55, 1.22)	0.44		
Dietary Intake Parameters				
**Fructose intake (g)	1.11 (1.00, 1.22)	0.04		
**Dietary fibre intake (g)	1.00 (0.61, 1.65)	0.93		
*Total fat intake (g)	1.11 (1.00, 1.22)	0.20		
*Saturated fat intake (g)	1.11 (0.82, 1.49)	0.44		
Demographic and Clinical Parameters				
**Age (years)	1.35 (1.11, 1.65)	0.006	1.22 (0.99, 1.49)	0.07
Gender				
Female	1.00	0.004	1.00	0.01
Male	1.09 (1.03, 1.16)		1.07 (1.02, 1.13)	
[†]**Ethnicity**				
Caucasian	1.00	0.41		
Non-Caucasian	1.02 (0.97, 1.07)			
**Time post transplantation (years)	1.11 (0.67, 1.82)	0.66		
Pre-emptive transplantation				
No	1.00	0.79		
Yes	1.01 (0.94, 1.09)			
Previous episodes of acute rejection				
No	1.00	0.86		
Yes	1.01 (0.91, 1.12)			
**Dialysis vintage (years)	1.02 (0.97, 1.06)	0.15		
ICED	1.01 (0.92, 1.10)	0.85		
Presence of diabetes				
Non-diabetic	1.00	0.09		
NODAT	1.04 (0.99, 1.09)			
Pre-DM	1.04 (0.96, 1.12)			
**MAP (mmHg)	1.49 (1.11, 1.82)	0.006	1.35 (1.11, 1.82)	0.006
Smoking status				
Non-smoker	1.00	0.38		
Ex-smoker	0.98 (0.92, 1.05)			
Current smoker	1.02 (0.92, 1.14)			
Ex-smoker / Current smoker	0.97 (0.91, 1.04)			
Alcohol intake (units/week)	1.01 (0.99, 1.02)	0.38		
Use of statin				
No	1.00	0.13		
Yes	0.95 (0.90, 1.02)			
Use of calcineurin inhibitor				
No	1.00	0.008	1.00	0.06
Yes	1.15 (1.04, 1.28)		1.09 (0.99, 1.20)	
Use of adjunctive antiproliferative agents				
No				
Yes	1.00	0.91		
	1.01 (0.92, 1.10)			
Use of prednisolone				
No	1.00	0.41		
Yes	1.03 (0.96, 1.11)			
Adjusted R^2 from final model			47%	

[§]Results in the final multivariate regression model were presented.

[δ]Due to the logarithmic transformation of the outcome variable, exponential of the beta coefficient has been applied, and hence beta coefficient and its associated 95% CI have been transformed and reported in the form of "Ratio". For the categorical variable, the ratio of the outcome in each category was relative to the outcome in the baseline category. For the continuous variable, unless otherwise stated, the ratio was given for one-unit increase in the explanatory variable.

[∝]CI = Confidence Interval.

[†]For the purpose of statistical analysis, the ethnicity of patients classified as "Afro-Caribbean", "Asian" and "Others" was grouped as "Non-Caucasian", 78% "Caucasian" versus 22% "Non-Caucasian".

Abbreviations: eGFR=estimated Glomerular Filtration Rate; **FTI**=Fat Tissue Index; **HDL**=High-Density Lipoprotein; **hsCRP**=high-sensitivity C-Reactive Protein; **ICED**=Index of Coexistent Disease; **LDL**=Low-Density Lipoprotein; **LTI**=Lean Tissue Index**; MAP**=Mean Arterial Pressure; **NODAT**=New Onset Diabetes After Transplantation; **Pre-DM**=Presence of Diabetes Mellitus pre-transplantation; **WC**=Waist Circumference.

(*) Coefficients reported for a 10-unit increase in explanatory variable.

(**) Coefficients reported for a 100-unit increase in explanatory variable.

Table 4. Predictors of Endotoxemia (Endotoxin) in Kidney Transplant Recipients

	Univariate Analysis		Multivariate Analysis[§]	
	Ratio[δ] (95% CI[ᵅ])	*p*-value	Ratio[δ] (95% CI[ᵅ])	*p*-value
Laboratory Parameters				
**Adiponectin (μg/mL)	0.74 (0.50, 1.10)	0.12		
**Vitamin D (nmol/L)	0.82 (0.74, 0.89)	<0.001	0.90 (0.82, 0.97)	<0.001
**Urate (μmol/L)	1.04 (1.00, 1.11)	0.07		
**eGFR (mL/min)	1.01 (0.90, 1.11)	0.96		
*HDL (mmol/L)	0.51 (0.32, 1.22)	0.006		
*LDL (mmol/L)	1.67 (1.27, 2.20)	<0.001		
*Total Cholesterol (mmol/L)	1.42 (1.12, 1.79)	0.004		
Triglycerides (mmol/L)	1.08 (1.06, 1.10)	<0.001	1.06 (1.04, 1.08)	<0.001
Anthropometric Measurements and Body Composition Parameters				
**WC (cm)	1.22 (1.11, 1.49)	0.004	1.22 (1.11, 1.35)	0.01
**FTI (kg/m^2)	1.65 (1.11, 2.46)	0.02		
*LTI (kg/m^2)	0.89 (0.84, 0.96)	0.002	0.95 (0.90, 1.00)	0.07
**Volume Expansion (%)	0.90 (0.61, 1.22)	0.40		
Dietary Intake Parameters				
**Fructose intake (g)	1.11 (1.02, 1.22)	<0.001	1.11 (1.01, 1.21)	0.01
**Dietary fibre intake (g)	0.90 (0.61, 1.35)	0.55		
*Total fat intake (g)	1.00 (0.90, 1.11)	0.76		
*Saturated fat intake (g)	1.09 (1.06, 1.12)	0.04		
Demographic and Clinical Parameters				
**Age (years)	0.82 (0.74, 1.00)	0.09		
Gender				
Female	1.00	0.87		
Male	1.01 (0.95, 1.06)			
[†]**Ethnicity**				
Caucasian	1.00	0.21		
Non-Caucasian	0.98 (0.94, 1.01)			
**Time post transplantation (years)	0.90 (0.61, 1.35)	0.63		
Pre-emptive transplantation				
No	1.00	0.08		
Yes	0.97 (0.92, 1.04)			
Previous episodes of acute rejection				
No	1.00	0.85		
Yes	1.01 (0.91, 1.08)			
ICED	1.02 (0.94, 1.09)	0.70		
Presence of diabetes				
Non-diabetic	1.00	0.09	1.00	0.08
NODAT	1.04 (0.98, 1.10)		1.03 (0.99, 1.06)	
Pre-DM	1.03 (0.99, 1.08)		1.03 (0.99, 1.08)	
**MAP (mmHg)	1.11 (0.90, 1.49)	0.21		
**Dialysis vintage (years)	1.01 (0.98, 1.09)	0.09		
Smoking status				
Non-smoker	1.00	0.31		
Ex-smoker	0.96 (0.91, 1.02)			
Current smoker	1.05 (0.96, 1.15)			
Ex-smoker / Current smoker	0.98 (0.93, 1.04)			
Alcohol intake (units/week)	0.99 (0.98, 1.00)	0.14		
Use of statin				
No	1.00	0.56		
Yes	1.02 (0.96, 1.07)			
Use of calcineurin inhibitor				
No	1.00	0.75		
Yes	1.01 (0.93, 1.11)			
Use of adjunctive antiproliferative agents				
No				
Yes	1.00	0.69		
	1.02 (0.94, 1.10)			
Use of prednisolone				
No	1.00	0.87		
Yes	0.99 (0.94, 1.06)			
Adjusted R^2 from final model			46%	

[§]Results in the final multivariate regression model were presented.

[δ]Due to the logarithmic transformation of the outcome variable, exponential of the beta coefficient has been applied, and hence beta coefficient and its associated 95% CI have been transformed and reported in the form of "Ratio". For the categorical variable, the ratio of the outcome in each category was relative to the outcome in the baseline category. For the continuous variable, unless otherwise stated, the ratio was given for one-unit increase in the explanatory variable.

[ᵅ]CI = Confidence Interval.

[†]For the purpose of statistical analysis, the ethnicity of patients classified as "Afro-Caribbean", "Asian" and "Others" was grouped as "Non-Caucasian", 78% "Caucasian" versus 22% "Non-Caucasian".

Abbreviations: **eGFR**=estimated Glomerular Filtration Rate; **FTI**=Fat Tissue Index; **HDL**=High-Density Lipoprotein; **ICED**=Index of Coexistent Disease; **LDL**=Low-Density Lipoprotein; **LTI**=Lean Tissue Index; **MAP**=Mean Arterial Pressure; **NODAT**=New Onset Diabetes After Transplantation; **Pre-DM**=Presence of Diabetes Mellitus pre-transplantation; **WC**=Waist Circumference.

(*) Coefficients reported for a 10-unit increase in explanatory variable.

(**) Coefficients reported for a 100-unit increase in explanatory variable.

Figure 1. Association between hsCRP and Endotoxin Levels in Kidney Transplant Recipients

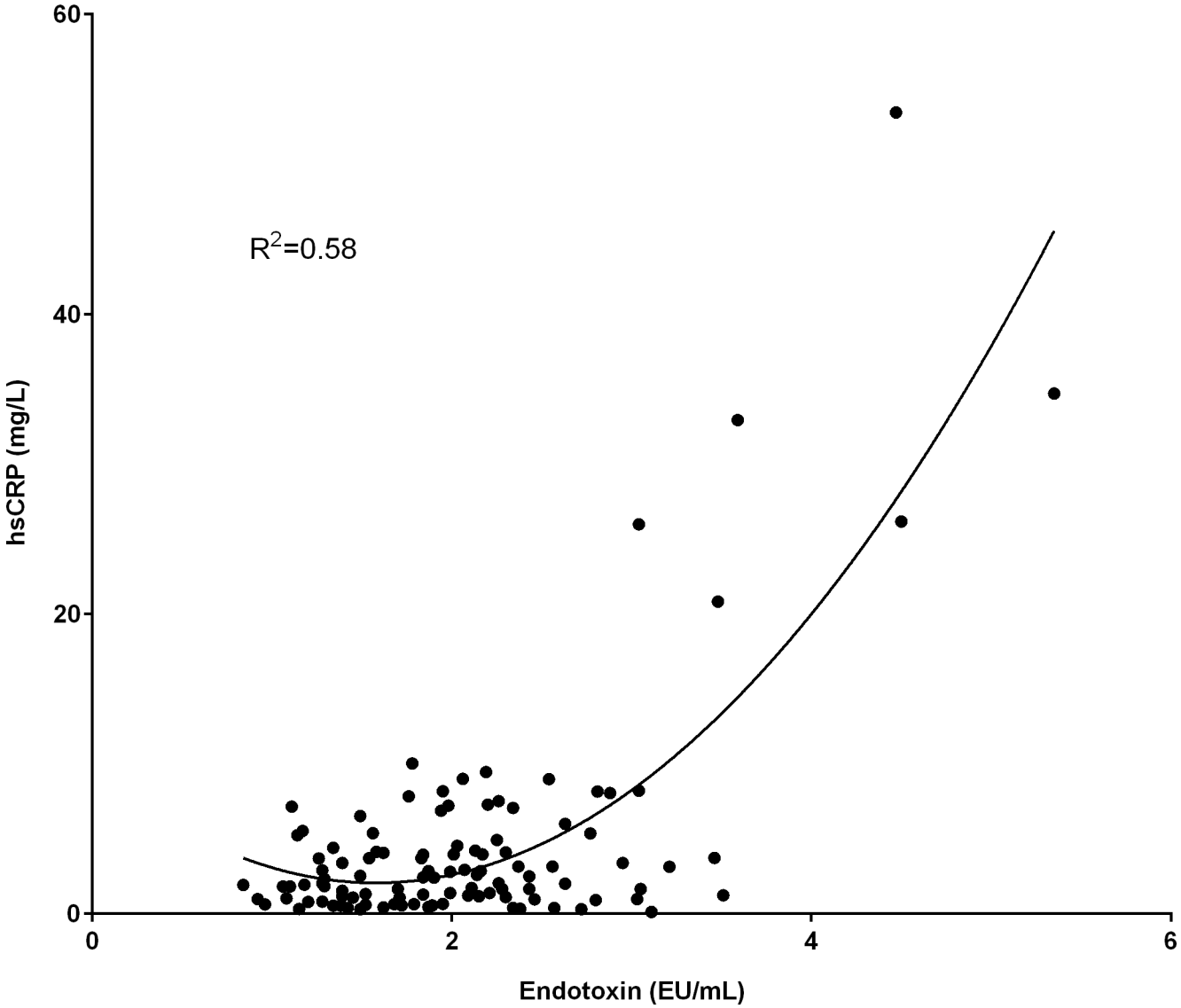


Figure 2a. Association between sE-selectin and Endotoxin Levels in Kidney Transplant Recipients

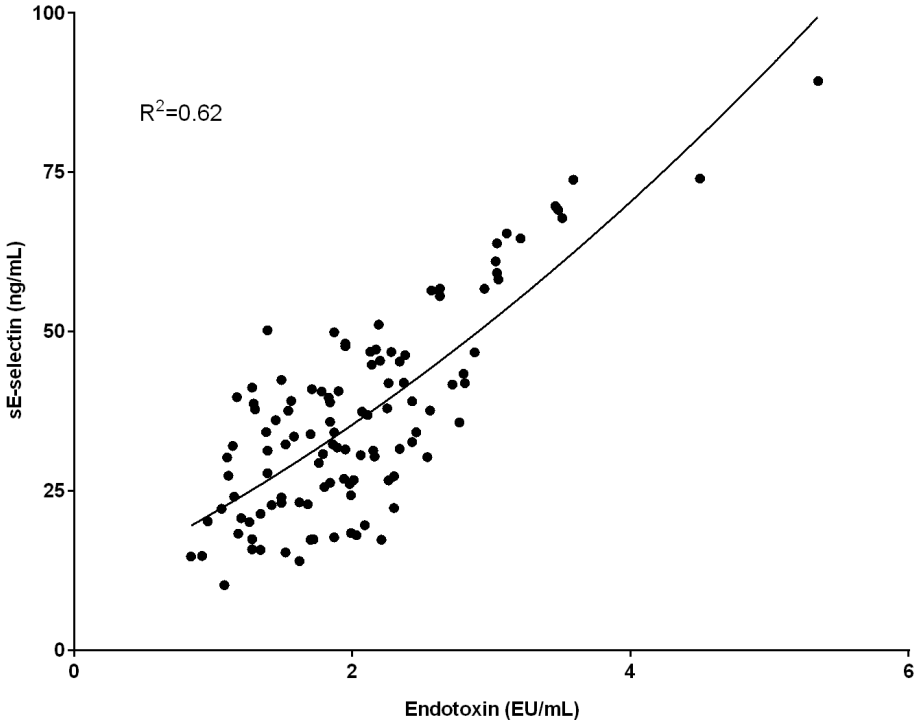


Figure 2b. Association between sE-selectin and hsCRP Levels in Kidney Transplant Recipients

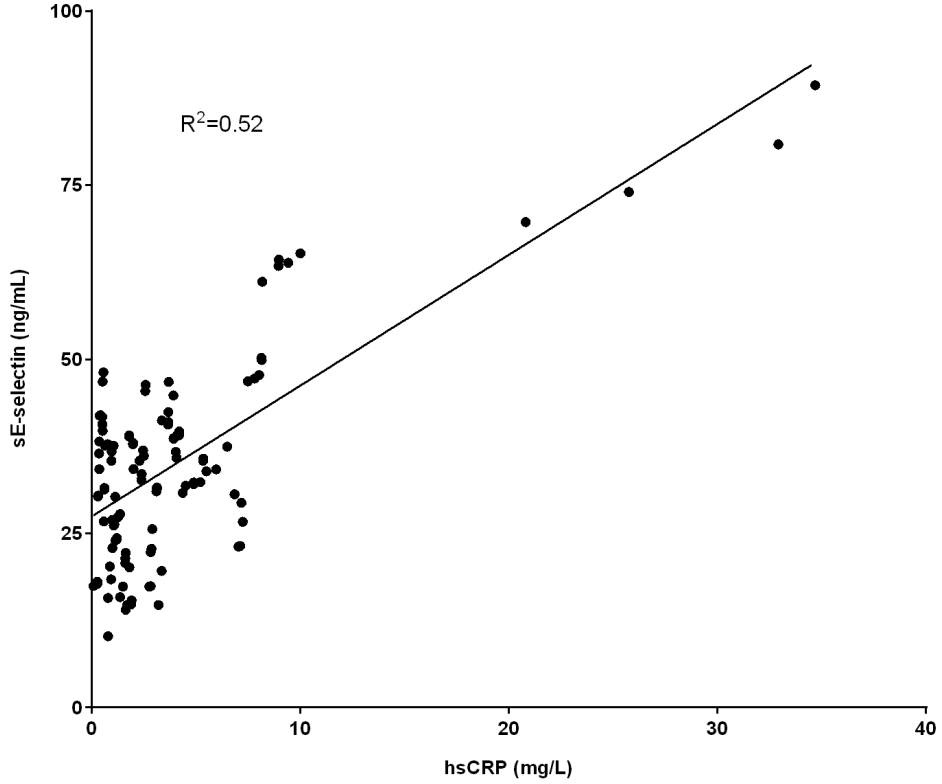
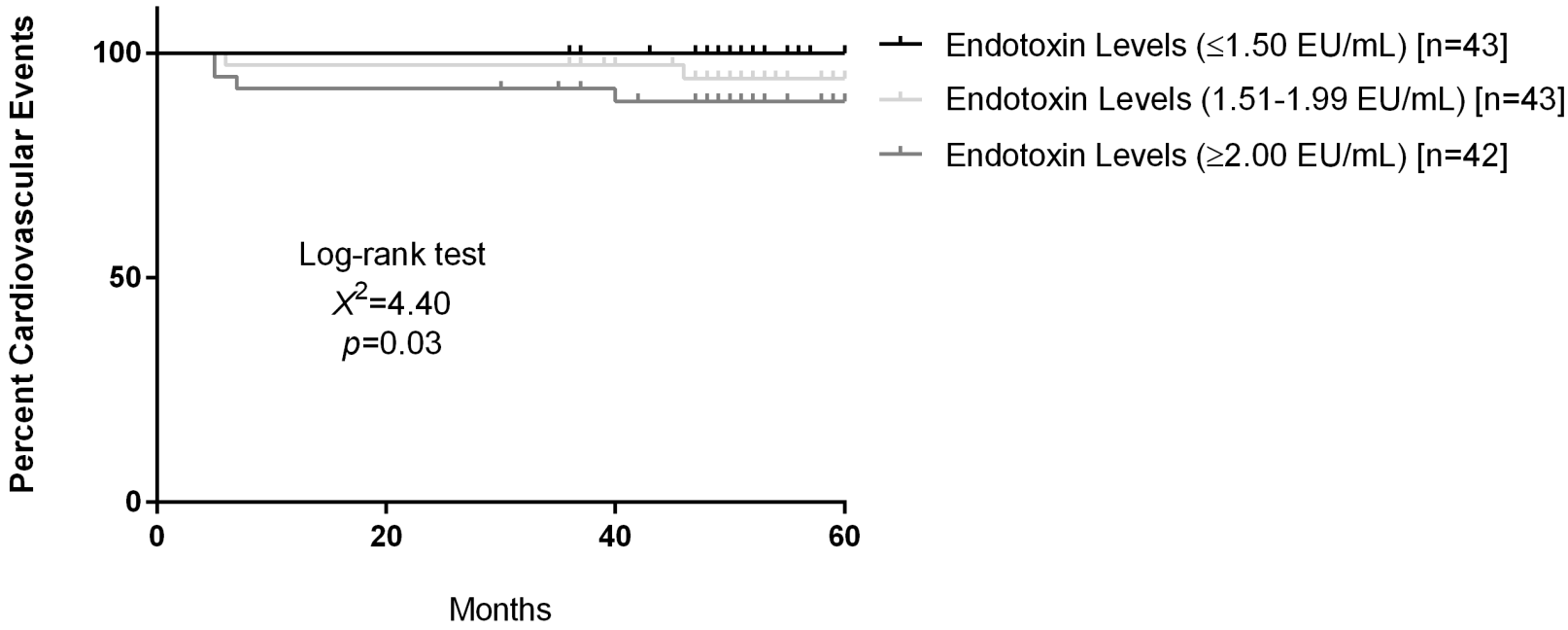
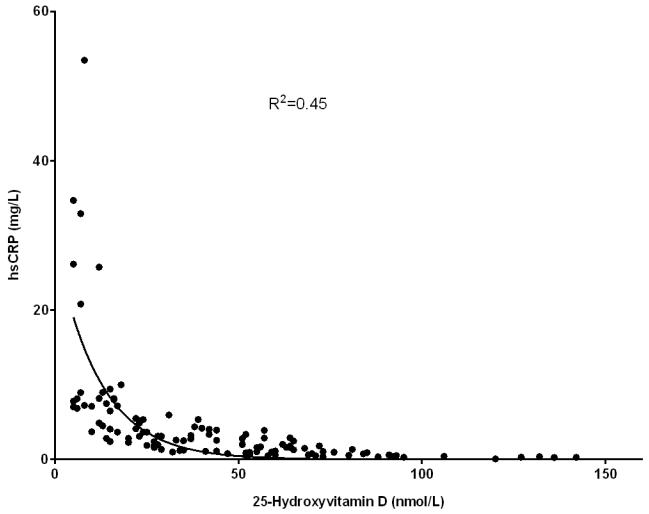


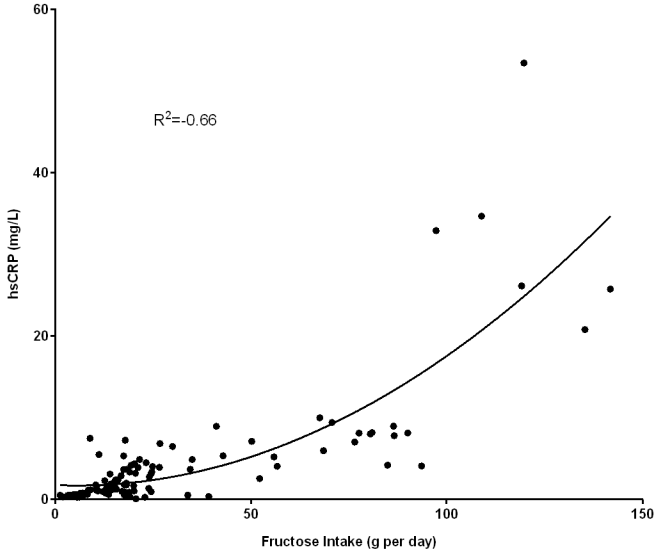
Figure 3. Kaplan-Meier Estimates of Time-to-Cardiovascular Event in Kidney Transplant Recipients with Different Levels of Endotoxemia by Tertile



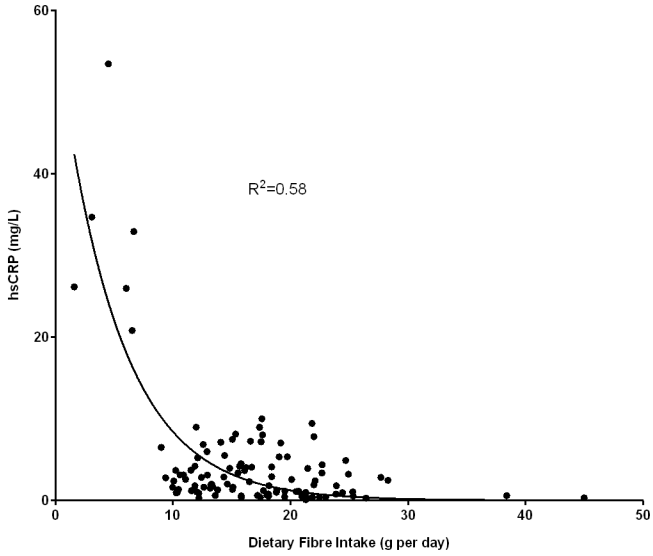
Supplementary Material, Figure S1a. Association between hsCRP and 25-Hydroxyvitamin D Levels in Kidney Transplant Recipients



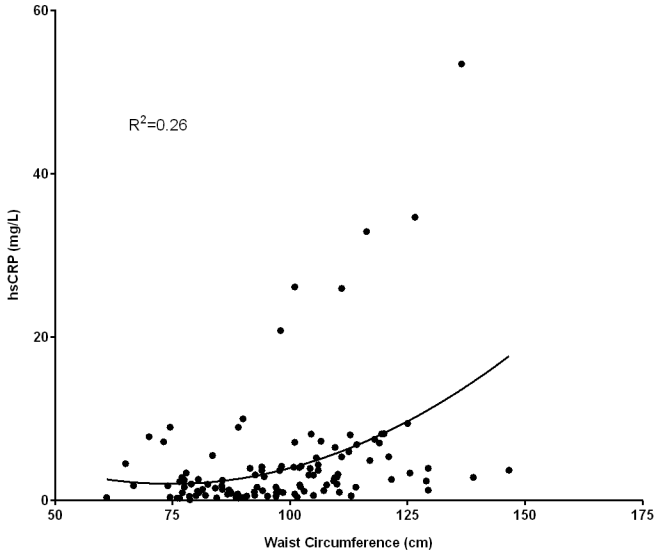
Supplementary Material, Figure S1b. Association between hsCRP Levels and Fructose Intake in Kidney Transplant Recipients



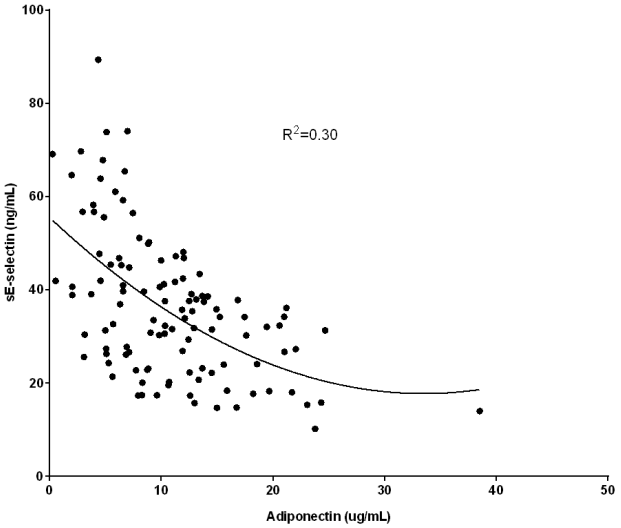
Supplementary Material, Figure S1c. Association between hsCRP Levels and Dietary Fibre Intake in Kidney Transplant Recipients



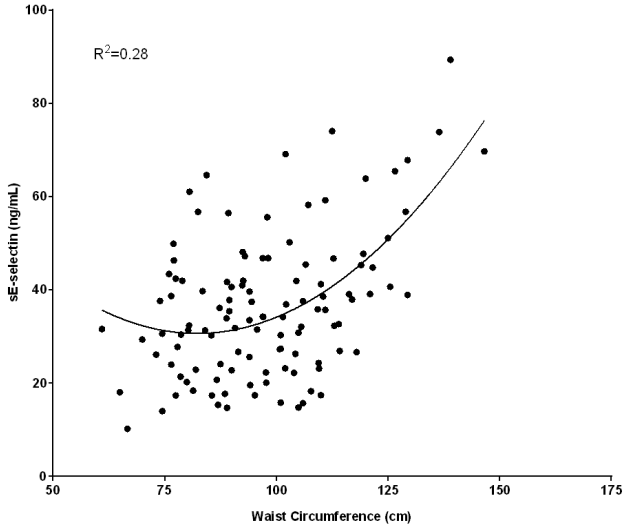
Supplementary Material, Figure S1d. Association between hsCRP Levels and Waist Circumference in Kidney Transplant Recipients



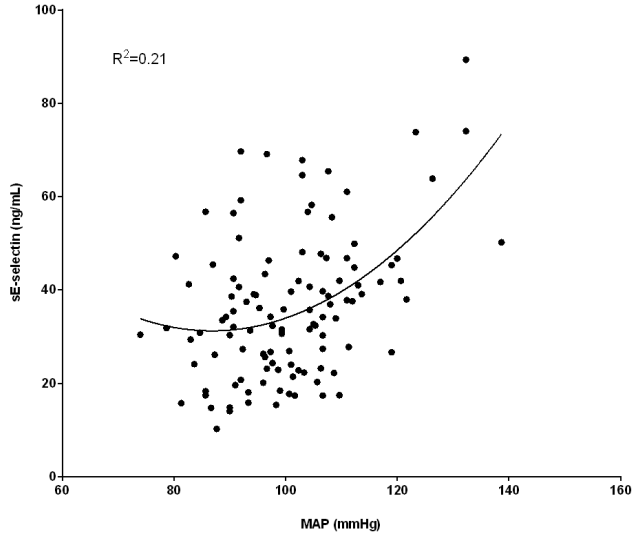
Supplementary Material, Figure S2a. Association between sE-selectin and Adiponectin Levels in Kidney Transplant Recipients



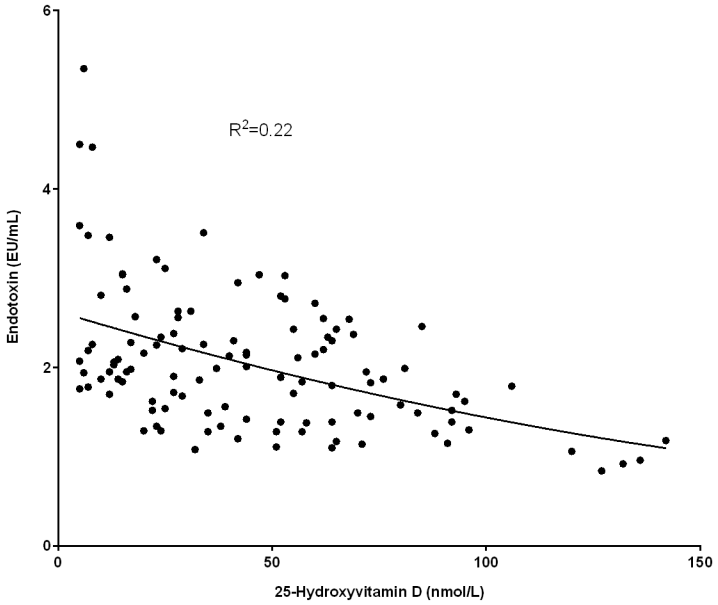
Supplementary Material, Figure S2b. Association between sE-selecin Levels and Waist Circumference in Kidney Transplant Recipients



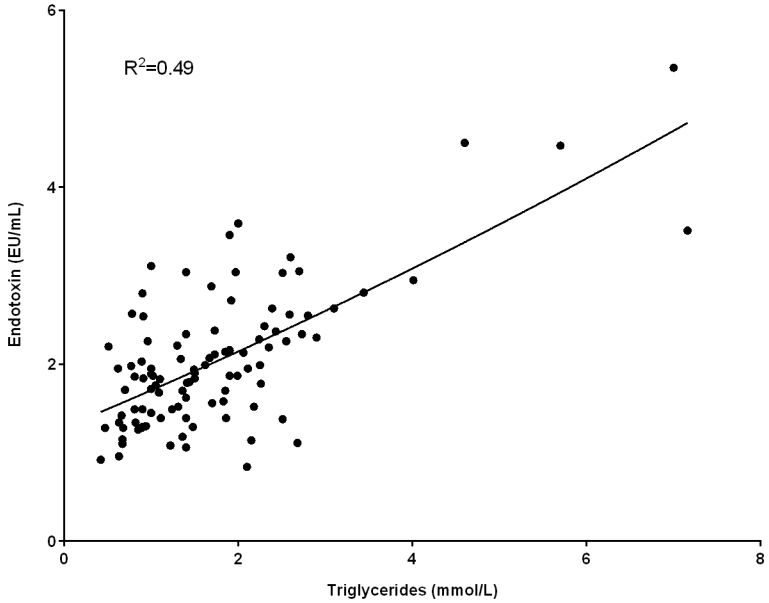
Supplementary Material, Figure S2c. Association between sE-selectin Levels and Mean Arterial Pressure (MAP) in Kidney Transplant Recipients



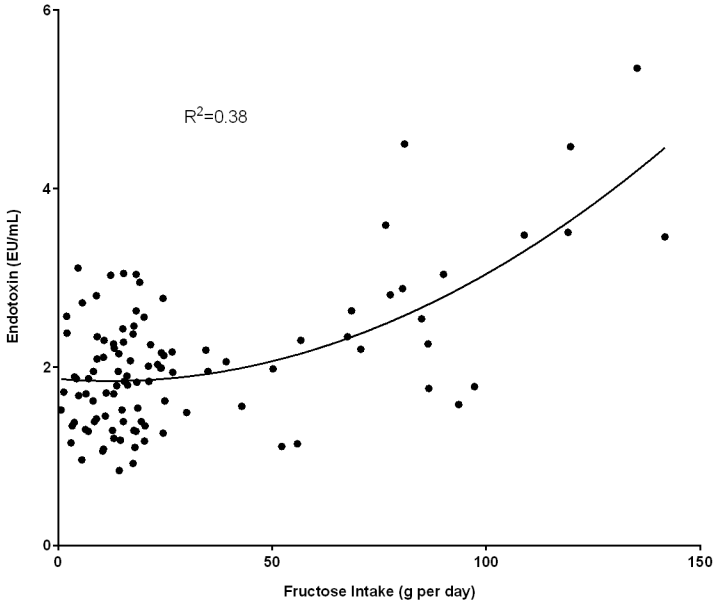
Supplementary Material, Figure S3a. Association between Endotoxin and 25-Hydroxyvitamin D Levels in Kidney Transplant Recipients



Supplementary Material, Figure S3b. Association between Levels of Endotoxin and Triglyceride Levels in Kidney Transplant Recipients



Supplementary Material, Figure S3c. Association between Endotoxin Levels and Fructose Intake in Kidney Transplant Recipients



Supplementary Material, Figure S3d. Association between Endotoxin Levels and Waist Circumference in Kidney Transplant Recipients