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Preoperative dietary intake of low-dose sulforaphane induces no clinically significant effect in living donor kidney transplantation

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ABSTRACT

Introduction: Sulforaphane (SFN) has anti-inflammatory properties, and is found in broccoli sprouts. Studies suggest that it protects against disease due to its anti-inflammatory activity. The impact of SFN on healthy people undergoing a surgical procedure has not been investigated.
Objective: To explore the effect of SFN in living kidney donors on the postoperative inflammatory response and recovery.
Methods: We performed a double-blind randomised controlled trial where donors followed a SFN-enriched (8 mg) preoperative diet.
Results: A total of 42 donors were included, there were no significant differences at baseline. Postoperative inflammatory response was consistent among both arms and subjective recovery showed no significant difference. Findings regarding postoperative kidney function suggest no consistently significant impact.
Discussion: A well-defined SFN-enriched diet did not have anti-inflammatory or a clinically relevant effect on the outcome. Due to the complexity of dietary modification of the inflammatory response, additional research is needed.

1. Introduction

Sulforaphane (SFN) is an isothiocyanate that is produced through the conversion of glucoraphanin, the primary glucosinolate found in the cruciferous vegetable broccoli (Juge et al., 2007). Glucoraphanin is present in high concentrations in broccoli seedlings but dilutes as the seedling matures into a full-grown broccoli plant (Juge et al., 2007; Tortorella et al., 2014; Zanini et al., 2014; Folkard et al., 2014; Medina et al., 2015). The consumption of seedlings facilitates the conversion of glucoraphanin to SFN. Several studies have suggested that the intake of SFN-rich cruciferous vegetables can protect against carcinogenesis and various chronic diseases due to the anti-inflammatory properties of SFN (Juge et al., 2007; Tortorella et al., 2014; Zanini et al., 2014; Folkard et al., 2014; Medina et al., 2015). SFN has been shown to activate the NRF2-mediated oxidative stress response, inducing a protective effect on oxidative damage, and interfere with toll-like receptor 4 and NF-kB signalling pathways. The NRF2 pathway in particular, is known to be upregulated following SFN administration (Chen et al., 2009; Zakkar et al., 2009; Kim et al., 2012; Meijer et al., 2015). Furthermore, broccoli consumption has been found to reduce the expression of markers associated with endothelial inflammation, such as intercellular cell adhesion molecules (ICAM) and vascular cell adhesion molecules (VCAM) (Chen et al., 2009; Zakkar et al., 2009; Kim et al., 2012). Despite these promising findings, the effects of SFN have not been extensively studied

Abbreviations: ESRD, End-stage renal disease; SFN, Sulforaphane; ICAM, ntercellular Cell Adhesion Molecules; VCAM, Vascular Cell Adhesion Molecules; CRP, C-Reactive Protein; NSAIDs, Nonsteroidal Anti-Inflammatory Drugs; IL-6, Interleukin 6; TNF-α, Tumour Necrosis Factor alpha; IFN-γ, Interferon Gamma; IL-10, Interleukin 10; NRF2, Nuclear factor erythroid 2–related factor 2; VAS, Visual Analog Score; MFI-20, Multidimensional Fatigue Inventory; SF-36, 36-Item Short Form Survey; MRA, Magnetic resonance angiography; CTA, Computed tomography angiography; HAL, Hand-Assisted Laparoscopy; DGF, Delayed graft function; AR, Acute rejection; LMM, Linear mixed-effects model; eGFR, Estimated Glomerular Filtration Rate.

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in humans, especially in the context of surgical procedures.

End-stage renal disease (ESRD) is a major cause of morbidity and mortality, necessitating kidney transplantation as a curative treatment (Ojo et al., 1994; Port et al., 1993; Schnuelle et al., 1998; Suthanthiran and Strom, 1994). Living kidney donation programs have demonstrated success in improving both the quality and quantity of life for recipients (Hariharan et al., 2000; Port et al., 2005), while having little impact on the quality of the donor (Lentine et al., 2019; Lentine et al., 2021; Poggio et al., 2021; Hariharan et al., 2021). However, kidney transplantation is accompanied by ischemia–reperfusion injury of the transplanted organ, which generates free oxygen radicals, resulting in oxidative stress and subsequent local and systemic inflammation, posing a risk to the donor, transplanted organ and recipient (Devarajan et al., 2003; Fletcher et al., 2009; Jang et al., 2009; Perico et al., 2004). Despite the recognized detrimental effects of oxidative stress during transplantation, there are currently no satisfactory treatments to effectively reduce its levels.

Living kidney donors represent an ideal study population for investigating the potential benefits of SFN as an added dietary intake. They must be in good health, the surgery is scheduled electively and laboratory measurements including the inflammatory response is acquired routinely, thereby minimising the burden on study participants. By investigating the effects of broccoli sprout consumption on markers of the postoperative inflammatory response and postoperative recovery in participants undergoing living kidney donation and their recipients, we aim to explore the therapeutic potential of SFN. This research will help determine whether an increased intake of SFN-rich cruciferous vegetables can provide protection against the inflammatory response associated with surgery and contribute to improved outcomes. In this study, we will examine the effects of adding broccoli sprouts, a rich source of SFN, to a short preoperative diet in healthy individuals undergoing living donor nephrectomy. We will focus on assessing markers of the acute phase response, such as leukocytes, CRP, and pro-inflammatory cytokine concentrations and markers indicating activation of the NRF2-mediated protective stress response. By evaluating the impact of SFN intake on these markers, and on postoperative recovery and kidney function, we may gain insights into the potential protective effects of SFN.

2. Methods

This study included patients scheduled for laparoscopic living donor nephrectomy at the Erasmus MC, where approximately 150 procedures are performed annually (NTS jaarverslag, 2022). Inclusion criterion for the study was sufficient knowledge of the Dutch language. Exclusion criteria were age below 18, regular use of corticosteroid or nonsteroidal anti-inflammatory drugs (NSAIDs) that might affect the inflammatory response, and a recipient requiring heparin as a therapeutic anticoagulant. The latter to prevent bleeding due to the biopsy, after reperfusion of the transplanted kidney. Living kidney donors and their recipients were approached separately by the trial coordinator at the outpatient clinic of the department of Surgery of the Erasmus MC Transplant Institute, University Medical Center Rotterdam, between January 2016 and September 2017. The medical ethical committee of Erasmus MC has approved the study protocol, patient information files, consent procedures, and other study-related documents and procedures. The trial has been registered under medical ethical assessment numbers MEC-2015-452 and NL53890.078.15.

The primary endpoint was the effect of randomisation on protective stress response in the donor, measured by markers of the acute phase response as defined by serum leukocyte number, c-reactive protein (CRP), and cytokines (IL-6, TNF-α, IFN-γ, IL-10). Secondary endpoints included the NRF2 mediated stress response, subjective postoperative recovery via questionnaires (VAS, MFI-20, SF-36, EuroQol) and recovery of kidney function after donation or transplantation.

Study participants underwent the same preoperative screening and workup as non-participants opting for living kidney donation. The standard screening included laboratory tests, preoperative examinations by a nephrologist and a surgeon or physician assistant, magnetic resonance angiography (MRA) or computed tomography angiography (CTA) to evaluate kidney (vascular) anatomy, chest x-ray and electrocardiogram, renal ultrasonography (if indicated), and a renogram (if indicated). The surgical technique involved Hand-Assisted (HAL) or laparoscopic donor nephrectomy, depending on the anatomy and preference of the surgical team.

After obtaining informed consent, all study participants were randomly assigned to either Diet 1 or Diet 2 using an envelope system. Both groups received standardized food boxes (without SFN-containing products) for 1 meal (dinner) for 5 days and were instructed to avoid SFN-containing vegetables such as arugula, broccoli, sprouts, cabbages, cauliflower, garden cress, collards, horseradish, kale, kohlrabi, mustard, radish, rutabaga, turnip, wasabi, watercress, and daikon. The intervention group additionally consumed 8 g of broccoli sprouts minced in 250 mL water (BroccoCress, Koppert Cress BV, Monster, The Netherlands) daily for 5 preoperative days. This corresponded to a daily intake of 8 mg of SFN. Donors in the control group consumed 8 g of butter lettuce minced in 250 mL water during the 5 preoperative days and were therefore blinded to the active substance, ensuring unbiased responses in the questionnaires.

Blood samples were collected before the start of the diet, at 12 h preoperatively and daily until discharge. Samples were taken at approximately the same time each day to avoid circadian rhythm variations. The blood samples were analysed for leukocytes, CRP, and cytokines (IL-6, TNF-α, IFN-γ, IL-10, VCAM, and ICAM) according to local hospital protocols. Creatinine and estimated Glomerular Filtration Rate (eGFR) measurement was performed at screening, admittance, daily until discharge, at 6 – and 12 weeks after surgery and at yearly follow-up. Three renal biopsies were taken of the donor kidney after nephrectomy to assess NRF2 mediated stress response. Biopsies were taken at the end of the cold storage period (timepoint 1), at the end of the second warm ischemia before reperfusion (timepoint 2), and 10–15 min after reperfusion (timepoint 3). To determine activation of the NRF2 pathway, we determined the mRNA expression levels of NRF2 target genes NQO1, HMOX-1, UGT1A, GSR, and GSTK1 as well as IL-6 in kidney biopsies taken just before implantation of the kidney in the recipient. Each sample was tested in duplicate. Total RNA was isolated from in rnalater stored biopsies using Trizol reagent (Invitrogen, Breda, the Netherlands) and purified by DNase treatement (RQ1 RNase free DNase, Promega Benelux bv, Leiden, the Netherlands). 2 microgram of of total RNA was reverse transcribed to cDNA using random hexamer primers (Invitrogen, Breda, the Netherlands) and Superscript II RT (Invitrogen, Breda, the Netherlands). Quantitative real-time polymerase chain reaction (RT-PCR) was performed using an iCycler real-time PCR system (BioRad, California, USA) using SYBR green (Sigma-Aldrich, St. Louis, USA). GAPDH, B2M and ACTB were selected as housekeeping genes. The geometric mean was used to average the control genes.

Standardized questionnaires were used to assess subjective postoperative recovery of the donor; VAS (Delgado et al., 2018) scored the amount of pain and nausea on a scale from none to severe, EuroQol (Rabin and de Charro, 2001) measured the ability to perform daily tasks and assessed health outcome, SF-36 (Ware and Sherbourne, 1992) evaluated pain, the ability to perform daily tasks, and quality of life, and MFI-20 (Smets et al., 1995) assessed fatigue levels and work-related questionnaires, which measured the ability to perform daily tasks related to work. The VAS and EuroQol questionnaires were completed before surgery and at days 1, 2, 3, 7, and 14 after surgery. The SF-36, MFI-20, EuroQol, and work-related questionnaires were completed before surgery and at 14 days and 3 months after surgery.

Statistical analysis was performed using R version 4.0.3 or newer. A two-sided significance level of 0.05 was used for all primary and secondary analyses, unless otherwise stated. Statistical models were built according to current standards (Rizopoulos, 2023) to determine the effect of the intervention on the outcome, adjusting for relevant factors

such as age, sex or prior dialysis. Regression analysis assumptions, including linearity, homoscedasticity, and normality, were visually checked. Clinical outcomes such as the incidence of delayed graft function (DGF) and acute rejection (AR) were compared as proportions. Linear mixed-effects models (LMM) were constructed to examine the effect of the intervention over time on postoperative kidney function. The models incorporated confounders such as age, sex and an interaction term between time and group. Due to missing values in follow-up, we performed multiple imputation and employed two distinct multiple imputation methods: we actively imputed creatinine with the function 2l.norm, a linear two-level model with homogeneous within group variances (Schafer and Yucel, 2002), and we passively imputed the associated eGFR.

3. Results

In this study, 111 donors were screened for eligibility. Among them, 11 individuals were found to be ineligible due to logistical reasons, and an additional donor was deemed ineligible due to recipient-related reasons. Consequently, 99 donors met the eligibility criteria and were approached by the trial coordinator. Ten individuals declined to participate, 6 citing concerns about the burden of the study, 1 participant expressed objections related to the dietary intervention, and 3 donors had objections to the study procedures. Furthermore, 47 individuals gave no response after being approached for participation. As a result, a total of 42 donors were ultimately included in the study. Among these 42 participants, 20 individuals were randomised to the control and 22 participants were allocated to the intervention arm. During the course of the study, five participants dropped out, all before having started the preoperative diet, leaving 17 donors available for data analysis in the control arm and 20 in the intervention arm, as seen in Fig. 1. Additional baseline & preoperative measurements are provided in Appendix A, Supplementary Table 1.

Table 1 shows the relevant baseline characteristics. The analysis showed that there was no difference in age between the control and intervention groups (58.1 and 52.8 years respectively). Similarly, there was no statistically significant difference in sex (52.9% and 75.0% respectively). Additionally, there was no difference in age at transplantation, sex of the recipient, side of kidney donation or transplantation, technique, surgery duration or prior dialysis.

Linear models were used to examine the changes due to the intervention in weight, BMI, creatinine and eGFR in donors. We compared the moment of inclusion and admittance, thereby reliably investigating a potential effect due to the diet. Every model showed no statistically significant effect due to the intervention ($p = 0.595$, 0.503, 0.197 and 0.218 respectively). Regarding length of hospital stay, a linear model including age, sex, and randomisation showed that none had a statistically significant effect. Chi-Square tests also showed no significant

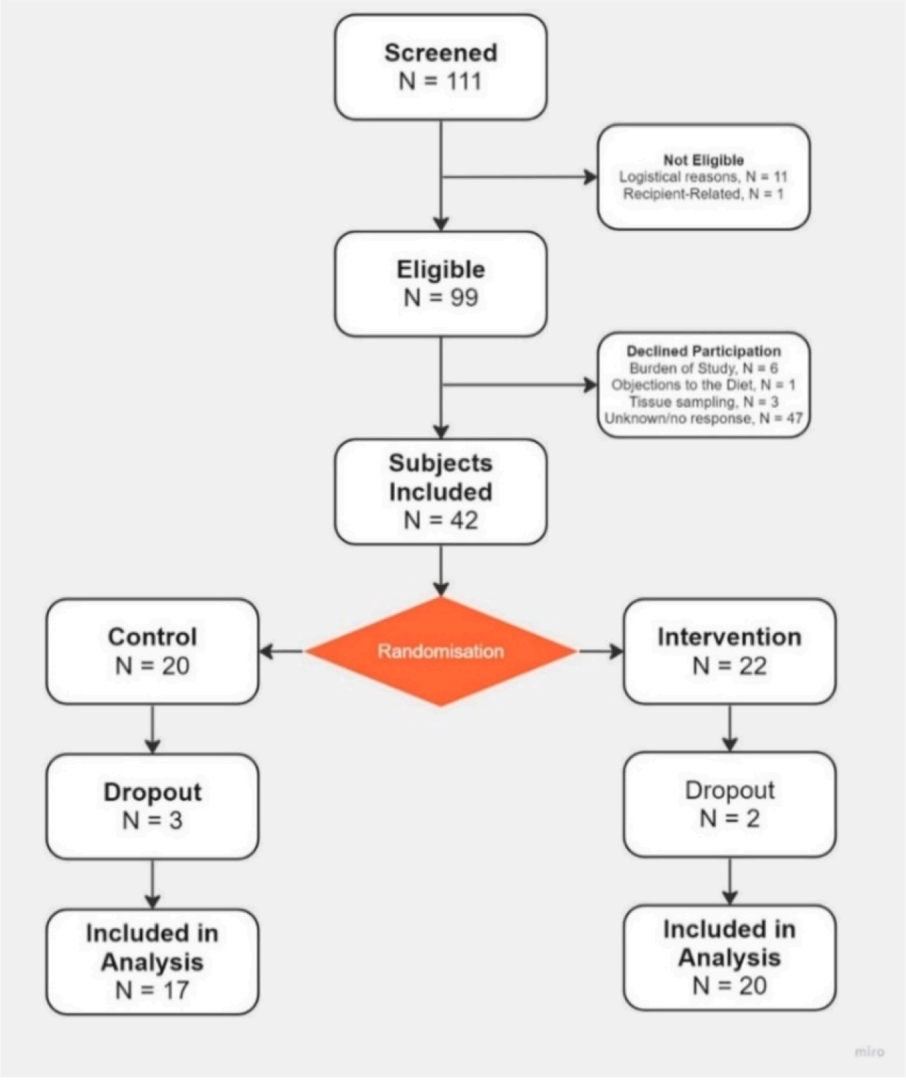

Fig. 1. Flowchart of recruitment and study participants. Legend: this figure presents the flowchart of study participants.

Table 1
Baseline Characteristics.

	Control (N = 17)	Intervention (N = 20)	Total (N = 37)
Donor Age			
Mean (SD)	58.1 (12.2)	52.8 (8.5)	55.2 (10.6)
Range	29.3–79.6	37.4–74.1	29.3–79.6
Donor Sex			
Female	9 (52.9%)	15 (75.0%)	24 (64.9%)
Male	8 (47.1%)	5 (25.0%)	13 (35.1%)
Recipient Age			
Mean (SD)	57.1 (18.3)	49.7 (15.6)	53.1 (17.1)
Range	12.0–77.1	17.8–73.5	12.0–77.1
Recipient Sex			
Female	7 (41.2%)	7 (35.0%)	14 (37.8%)
Male	10 (58.8%)	13 (65.0%)	23 (62.2%)
Donation Side			
Left	15 (88.2%)	13 (65.0%)	28 (75.7%)
Right	2 (11.8%)	7 (35.0%)	9 (24.3%)
Surgery Method			
HAL	5 (29.4%)	8 (40.0%)	13 (35.1%)
Laparoscopic	12 (70.6%)	12 (60.0%)	24 (64.9%)
Surgery Time			
Mean (SD)	153 (30.8)	154 (52.0)	154 (43.0)
Range	97–232	74–249	74–249
Transplantation side			
Left	4 (23.5%)	3 (15.0%)	7 (18.9%)
Right	13 (76.5%)	17 (85.0%)	30 (81.1%)
Prior Dialysis			
No	10 (58.8%)	13 (65.0%)	23 (62.2%)
Yes	7 (41.2%)	7 (35.0%)	14 (37.8%)

Legend: this table presents the relevant baseline characteristics of both the Donor and Recipients. SD = Standard Deviation, HAL = Hand-Assisted Laparoscopic.

association between randomisation and rejection ($p = 0.9924$, control 29.4% and intervention 35.0%) and graft failure ($p = 1$, control 11.8% and intervention 10.0%).

We measured serum cytokine and adhesion molecule concentrations in 18 kidney donors in the intervention group and 15 control donors, until postoperative day 3. VCAM concentrations were comparable between the groups; concentration did not differ before and after the diet but was increased in both groups on all postoperative days (p for all $>$ 0.1). ICAM concentrations showed no change over time but were consistently significantly lower in the intervention group (p for all $>$ 0.01 and $<$ 0.05). Before the start of the diet, TNF-A concentration was higher in the SFN group due to an outlier but was low in both groups after the diet (p for all $>$ 0.1). On postoperative day 1, levels were equally increased in both groups, and returned to baseline on days 2 and 3 (p for all $>$ 0.1). Baseline IL-6 concentrations were low, and unchanged by the diet. IL-6 peaked on postoperative day 1 and was significantly lower in the intervention group ($p < 0.05$), as shown in Fig. 2.1–2.4. We found no statistically significant differences in the target genes NQO1, HMOX-1, GSR, UGT1A, GSTK1, and IL-6 in the acquired biopsies, at all three timepoints (p for all $>$ 0.1). See Fig. 3.1–3.6 for the graphical representation.

Linear models were constructed to assess the impact of the intervention on 2 domains of the SF-36 questionnaire: Physical Functioning and General Health. Comparisons were made between baseline and admittance, 2 weeks after surgery, and 3 months after surgery. Regarding physical functioning: the effect of randomisation was not significant in all three models; baseline vs admittance (estimate = −2.7294, $p = 0.2660$), baseline vs 2 weeks after surgery (estimate = −1.1197, $p = 0.897$), and baseline vs 3 months after surgery (estimate = 6.3678, $p = 0.147$). This model was corrected for the surgery technique, which also showed no statistically significant effect on the outcome. General health showed the same pattern: the effect of randomisation was not significant; baseline vs admittance (estimate = 1.15775, $p = 0.68$), baseline vs 2 weeks (estimate = −5.3995, $p = 0.1754$), and baseline vs 3 months (estimate = 7.2223, $p = 0.097$). This model was also corrected for the type of surgery, which also showed no statistically significant effect on the outcome. We additionally conducted a LMM analysis and in these models examined the effect of time (non-linear), age, and an interaction between time and surgery. The analysis included random effects for time nested within the grouping variable. The results showed that surgery had a significant negative effect on physical functioning, with an average decrease of 33.30 points ($p = <0.0001$). The interaction between time and surgery showed that the intervention reduced this negative effect by 8.36 points, although this was just shy of statistical significance (p-value = 0.0569). The general health analysis showed no significant effect of surgery or the interaction with time on general health scores. See Fig. 4 for the graphical representation of the mixed models.

We conducted an extensive LMM analysis for creatinine and eGFR in both the donor and recipient: for each we constructed two models. Each model included the (non-linear) effect of time, surgery, an interaction of time with surgery, and intervention and sex. The initial model also included a factor for randomisation at baseline, which corrects for any residual baseline difference. This is not preferred in the donor since randomisation was performed correctly and therefore might incorrectly bias the model. It is preferred in the recipients since they were not randomised but followed the randomisation of the donor. The random effects component of the models included random effects for time nested within the grouping variable. In every model we performed two analyses for the effect of the intervention: one over time and one for the effect at the moment of surgery.

The donor creatinine LMM, without the baseline correction, showed that the effect of randomisation over time was estimated at 6 points (95% CI: [2.86, 9.68], $p = 0.0006$) difference in creatinine levels between the control and intervention, where the control group had a higher creatinine. Sex was also found to be a significant factor: male donors had 31 points higher creatinine levels ($p < 0.001$). The interaction term was found to be statistically significant: the increase in creatinine due to surgery was reduced by 6.27 points (95% CI: [2.74, 12.53], $p = 0.0004$) by the intervention, thereby resulting in the difference over time. In the second model, with baseline correction, results were consistent except for the overtime analysis: it was estimated at 6 points (95% CI: [−0.76, 15.36], $p = 0.1517$) difference. The increase in creatinine due to surgery remained consistent with the initial model: it was reduced by 6.09 points (95% CI: [2.35, 9.85], $p = 0.0012$) by the SFN-Diet. When we performed the same analysis on eGFR of the donor, we found that the intervention did not lead to a statistically significant difference over time. The estimated difference in eGFR was 2.84 (95% CI: [−4.35, 1.36], $p = 0.915$) points lower for control. The interaction was also non-significant: 1.45 (95% CI: [−1.42, 4,41], $p = 0.306$). The second model with baseline correction confirmed that the intervention did not result in a statistically significant difference over time ($p = 1$). The estimated difference in eGFR at surgery was also statistically non-significant ($p = 0.43$).

Regarding creatinine levels in the recipient, we again constructed two models, with and without baseline correction for randomisation. The first model showed that the intervention did not lead to a statistically significant difference in creatinine levels over time for recipients who received a kidney from an intervention donor (9.9, 95% CI:

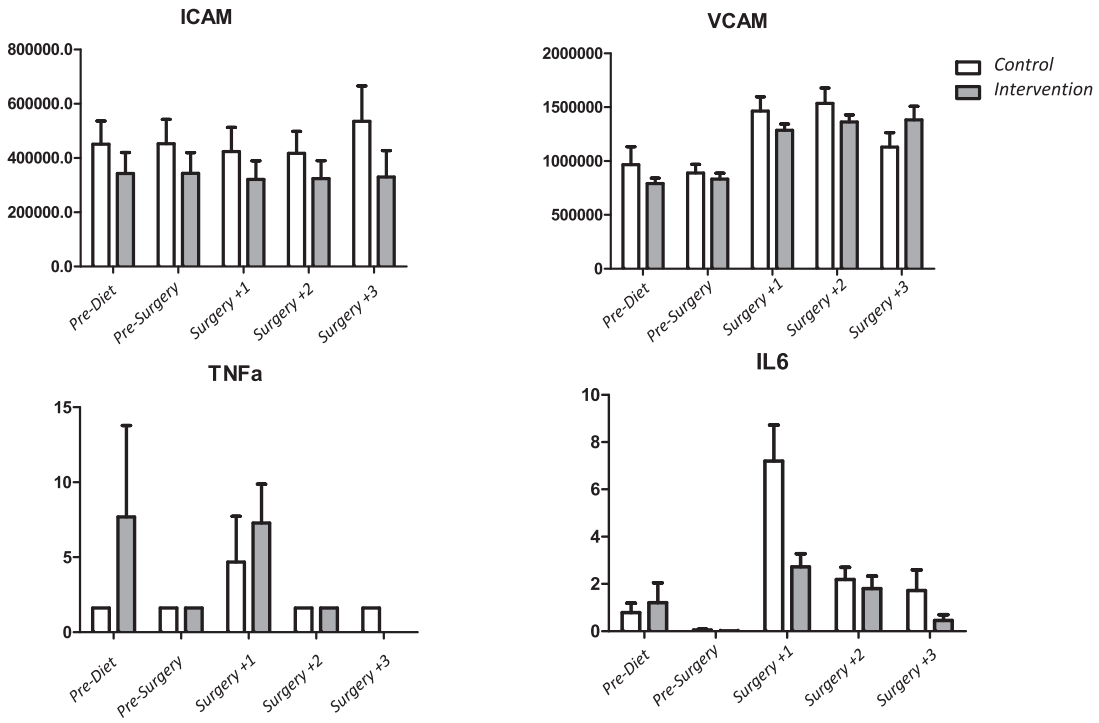

Fig. 2.1–2.4. Serum concentrations of ICAM, VCAM, TNF-A and IL-6 in the days after surgery. Legend: ICAM = intercellular cell adhesion molecules, VCAM = vascular cell adhesion molecules, TNFa = Tumour necrosis factor alpha, IL 6 = Interleukin 6. The error bars represent the 95% Confidence Interval.

[–23.78, 43.65, p = 1). When looking at the interaction, investigating the effect at the moment of surgery, this factor shows a statistically significant effect of −58.9 (95% CI: [−94,5, −3.46], p = 0.0006) points creatinine for a recipient of an intervention kidney. When performing the same analysis in a model without the baseline correction, we found that the effect over time was 35.85 (95% CI: [10.82, 60.86], p = 0.025) points lower creatinine for a recipient who received a kidney from an SFN-donor. Additionally, the interaction term was consistently statistically significant with an effect of −35.85 (95% CI: [−60.86, −10.83], p = 0.005). Both analyses were corrected for age and if recipients had been on dialysis before transplantation. When we performed the same analysis for eGFR, starting with the effect of the intervention over time, we found no statistically significant difference. The estimated difference was 6.28 (95% CI: [−3.23, 12.60], p = 0.0515) points higher eGFR for a recipient who received a kidney from an SFN-donor. The interaction term was also non-significant; 3.48 higher eGFR for a recipient of an intervention kidney (95% CI: [−0.24, 7.22], p = 0.0619). In this initial model, the baseline correction of randomisation was not statistically significant (p = 0.4509). In the model without baseline correction, we found that the intervention produced a statistically significant difference 4.13 (95% CI: [0.96, 7.31], p = 0.0107). This LMM consistently showed that the effect of surgery was mitigated by the intervention: the interaction was statistically significant with an effect of 4.13 higher eGFR for a recipient of an intervention kidney (95% CI: [1.81, 6.46], p = 0.0108), thereby resulting in the difference over time. See Figs. 5–10, for the graphical representation of the mixed models.

Due to the selective missingness during follow-up due to the Covid19 Pandemic, we used Multiple Imputation to impute 25 databases with 75 iterations with creatinine via the function 2l.norm, yielding promising results that aligned well with the observed values. Second, we passively imputed eGFR using the actively imputed creatinine values. The eGFR values did not perfectly correspond to the mean observed value, which met expectations due to the moment of missingness considering the absence of measurements at the 4- and 5-year points following surgery due to the Covid19 Pandemic. The use of multiple imputation and following LMM analysis did not yield any substantial deviations or novel insights compared to previous analyses. See Appendix B, Figs. 1 and 2, for the graphical representation of the results of multiple imputation.

4. Discussion

In this study we aimed to explore the effect of dietary intake of SFN in living kidney donors on the postoperative inflammatory response, subjective recovery and kidney function. We found no consistent improvement in the inflammatory response, a small but non-significant increase in postoperative physical recovery and a significant but only small improvement in postoperative kidney function in both the donor and the recipient. Looking at our analysis, we conclude that randomisation was performed correctly, while having a relatively small sample size. The findings in the preoperative analysis show no significant influence of the diet on preoperative (post-intervention) weight, BMI, creatinine levels, and eGFR. There was no significant effect of the randomisation on the length of hospital stay, complications, rejection or graft failure. The results from the inflammatory markers suggest that the intervention diet did not have a consistent substantial impact on the measured cytokine and adhesion molecule concentrations or the expression of NRF2 target genes, albeit that IL-6 was significantly lower in the intervention group.

When focusing on the patient-reported postoperative recovery of the donor, the results consistently showed no significant effect of the SFN-enriched diet. Therefore, it can be concluded that the diet did not have a substantial impact on physical recovery. The LMM analysis on physical functioning showed that the intervention group experienced a relevant but just shy of statistical significance effect in reducing the negative impact of surgery. It seems that there is a tendency for the diet to have a positive impact on physical function during recovery. When focusing on postoperative kidney function of the donor, we found the expected impact donor nephrectomy had on creatinine levels. There was a small effect of the diet on creatinine trajectory, where the control group had a higher creatinine, attributed to a reduction in the increase in

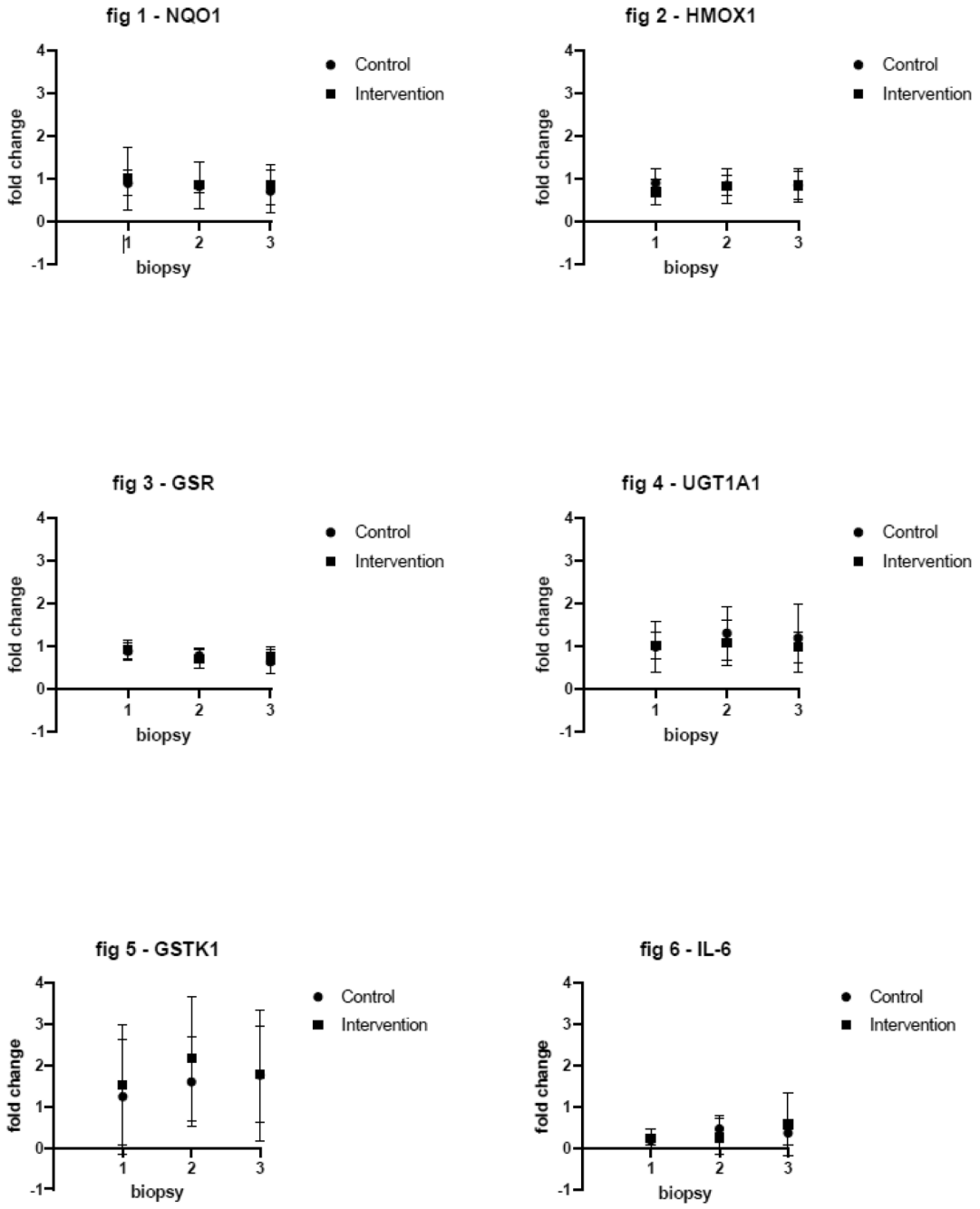

Fig. 3.1–3.6. mRNA expression of NRF2 target genes in kidney biopsies. Legend: Tissue samples were taken at the end of the cold storage period (1), before restoration of blood flow in the recipient (2), and 10–15 min after reperfusion (3). NQO1 = NAD(P)H quinone dehydrogenase 1, HMOX1 = heme oxygenase 1 gene, GSR = Glutathione-Disulfide Reductase, UGT1A1 = UDP glucuronosyltransferase 1 family, polypeptide A1, GSTK1 = Glutathione S-transferase kappa 1, IL-6 = Interleukin 6. The error bars represent the 95% Confidence Interval.

creatinine at the moment of surgery. When we performed the same analysis on trajectory of eGFR over time, we found the intervention did not lead to a statistically significant difference. Therefore, we concluded that the SFN-enriched diet did not have a clinically or statistically significant effect on the recovery of the kidney function of the donor. In the recipient these findings were less unambiguous: the SFN-enriched diet did not lead to a statistically significant difference in creatinine levels over time, when corrected for potential differences at baseline. However, when looking at the effect of the intervention at the moment of surgery, we saw a statistically significant effect in recipients of a donor who had received the SFN-enriched diet. When performing the same analysis in a model without the baseline correction for randomisation, we found that the effect over time and the effect at surgery were consistently significant. When we performed the same analysis for eGFR, we found that the intervention did not have a statistically significant effect. In a model without additional baseline correction, we did find a statistically significant difference in the eGFR- trajectory due to the intervention. Overall, our findings suggest that the intervention did not consistently significantly impact creatinine and/or eGFR levels over time, in both the donor and the recipient. Some analyses showed that the SNF-enriched diet reduced the negative effect of surgery on kidney function in the donor and had a positive effect on the kidney function in the recipient, but since these findings were not unambiguous, and the effect small, it bears little to no clinical significance.

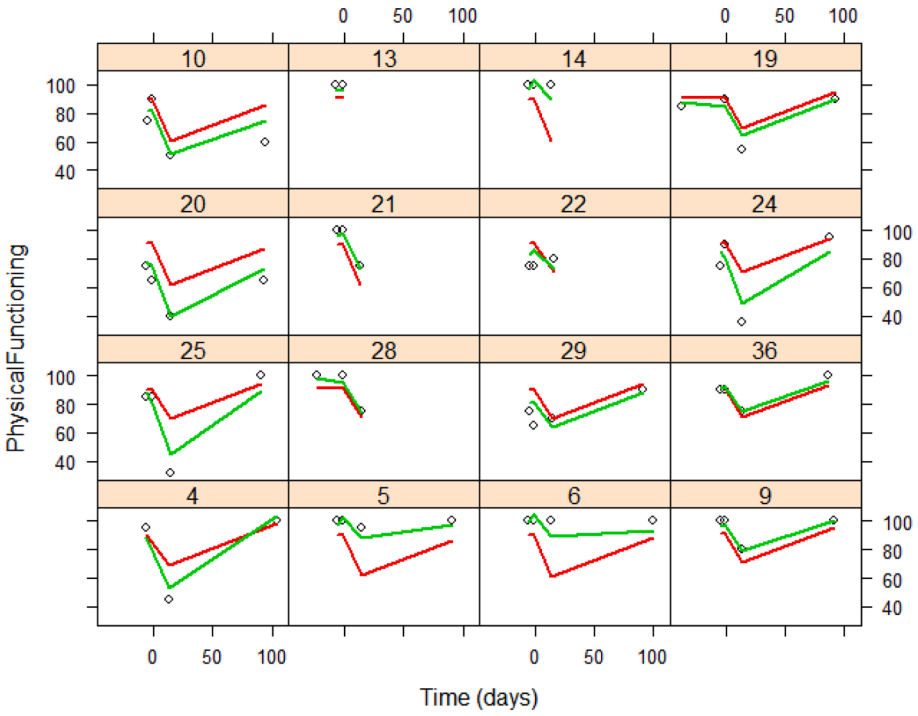

Fig. 4. Physical Functioning Score Donor, Descriptive. Legend: X-Axis: Time (days), Y-Axis: Physical Functioning score (1–100). All data represent Donor Physical Functioning score. Figs. 4–8 follow the same principles: (i) Time is in days, with 0 being the day of surgery. Measurements before are from the pre-donation or –transplantation workup, while measurements after are from during the hospital admittance or outpatient clinic follow-up. (ii) One block is one donor or recipient, and missing points are due to missingness of the outcome (laboratory measurements not performed or questionnaires not completed). (iii) The red line indicates the overall (mean) trajectory. (iv) The green line indicates the subject-specific trajectory.

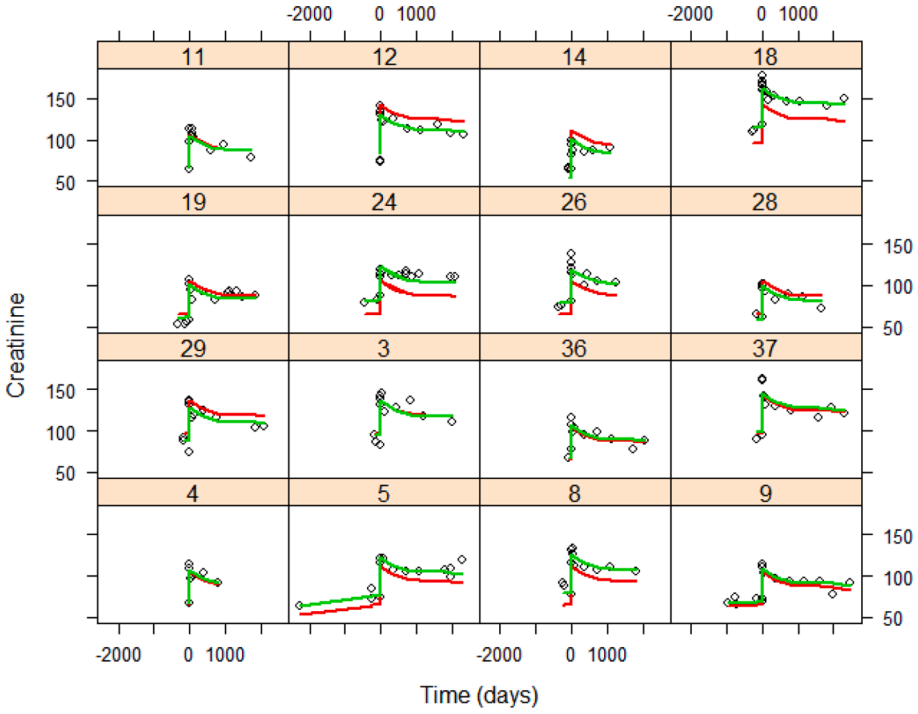

Fig. 5. Creatinine Concentration Donor, Descriptive. Legend: X-Axis: Time (days), Y-Axis: Creatinine serum concentration (μmol/L). Figs. 4–8 follow the same principles: (i) Time is in days, with 0 being the day of surgery. Measurements before are from the pre-donation or –transplantation workup, while measurements after are from during the hospital admittance or outpatient clinic follow-up. (ii) One block is one donor or recipient, and missing points are due to missingness of the outcome (laboratory measurements not performed or questionnaires not completed). (iii) The red line indicates the overall (mean) trajectory. (iv) The green line indicates the subject-specific trajectory.

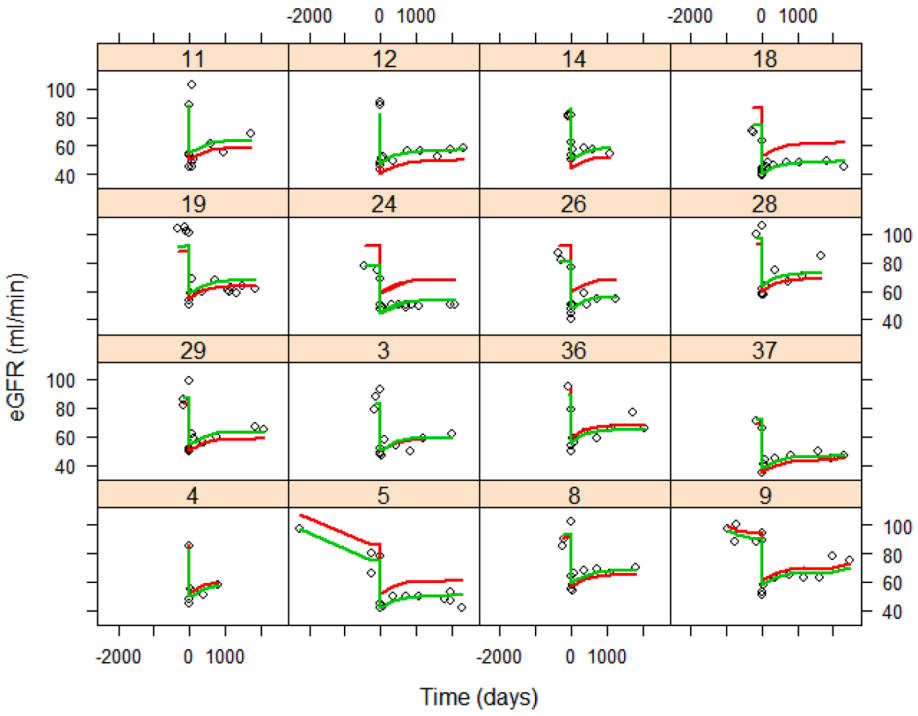

Fig. 6. eGFR Trajectory Donor, Descriptive. Legend: Axis: Time (days), Y-Axis: eGFR via CKD-EPI (ml/min). Figs. 4–8 follow the same principles: (i) Time is in days, with 0 being the day of surgery. Measurements before are from the pre-donation or –transplantation workup, while measurements after are from during the hospital admittance or outpatient clinic follow-up. (ii) One block is one donor or recipient, and missing points are due to missingness of the outcome (laboratory measurements not performed or questionnaires not completed). (iii) The red line indicates the overall (mean) trajectory. (iv) The green line indicates the subject-specific trajectory.

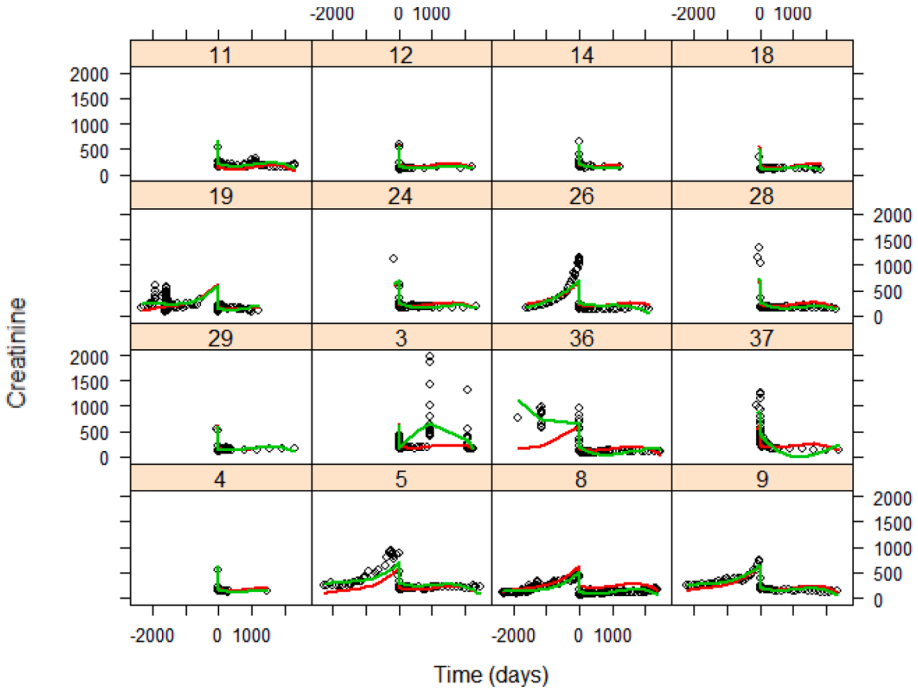

Fig. 7. Creatinine Concentration Recipient, Descriptive. Legend: X-Axis: Time (days), Y-Axis: Creatinine serum concentration (μmol/L). Figs. 4–8 follow the same principles: (i) Time is in days, with 0 being the day of surgery. Measurements before are from the pre-donation or –transplantation workup, while measurements after are from during the hospital admittance or outpatient clinic follow-up. (ii) One block is one donor or recipient, and missing points are due to missingness of the outcome (laboratory measurements not performed or questionnaires not completed). (iii) The red line indicates the overall (mean) trajectory. (iv) The green line indicates the subject-specific trajectory.

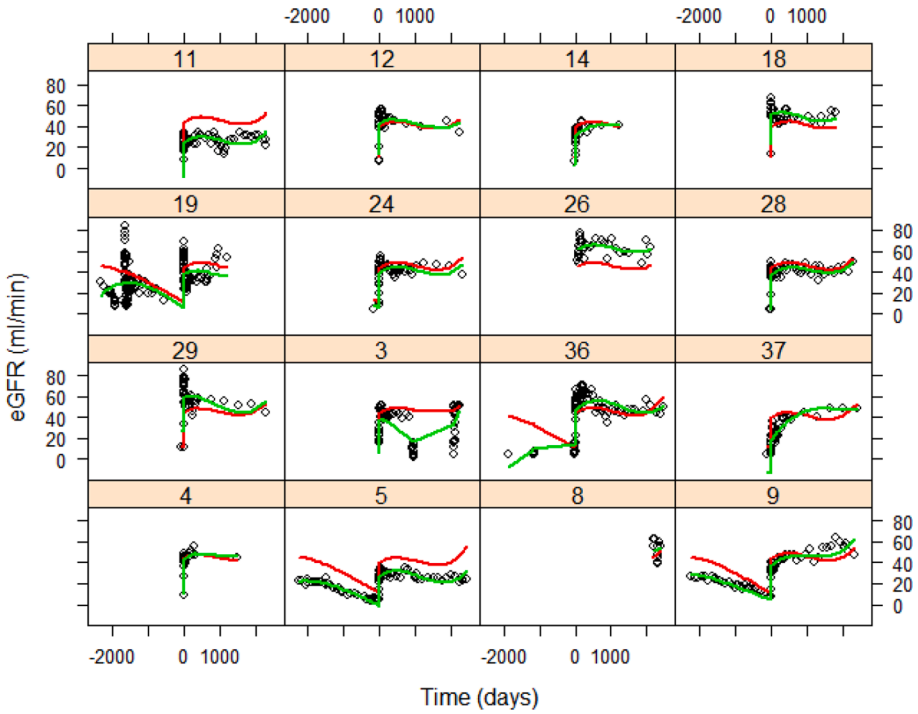

Fig. 8. eGFR Trajectory Recipient, Descriptive. Legend: Axis: Time (days), Y-Axis: eGFR via CKD-EPI (ml/min). Figs. 4–8 follow the same principles: (i) Time is in days, with 0 being the day of surgery. Measurements before are from the pre-donation or –transplantation workup, while measurements after are from during the hospital admittance or outpatient clinic follow-up. (ii) One block is one donor or recipient, and missing points are due to missingness of the outcome (laboratory measurements not performed or questionnaires not completed). (iii) The red line indicates the overall (mean) trajectory. (iv) The green line indicates the subject-specific trajectory.

Earlier studies identified sulforaphane as a bioactive compound with the potential to reduce inflammation via its anti-inflammatory properties (Axelsson et al., 2017; Chen et al., 2009; Zakkar et al., 2009; Kim et al., 2012; Meijer et al., 2015). Interestingly, other studies encompassing dietary preconditioning methods such as caloric and/or protein restriction found more relevant clinical outcomes, such as reducing the incidence of acute rejection and/or improving postoperative kidney function (Jongbloed et al., 2016; Jongbloed et al., 2020). Translating the effects of dietary preconditioning from preclinical to clinical studies has shown mixed results, with several studies not finding the same magnitude of effect (Grundmann et al., 2018; Kip et al., 2021; Kip et al., 2019). Although we did partly find the beneficial protective effect of sulforaphane as a dietary compound in our perioperative model, the magnitude was less than expected, resulting in just a small benefit in clinical outcomes. This could be due to several reasons, for instance the dosage of sulforaphane in the individual donors. We tried to alleviate this by measuring the concentration of the active compound (using methylene chloride extraction and reversed-phase chromatography), all confirming the calculated dose of 8 mg, but it could be that the dosage should be increased to improve the outcomes significantly. One could argue that, instead of increasing the dosage, the duration of the diet should be increased, thereby providing a more longitudinal exposure like other studies (Wang et al., 2022; Fahey and Kensler, 2021). We hypothesize that insufficient exposure is the most likely reason for not finding any clinical relevant outcomes in our trial, since we did not find statistically significant differences in NRF2 mediated stress response, which we did expect from earlier studies (Chen et al., 2009; Zakkar et al., 2009; Kim et al., 2012; Meijer et al., 2015). Another argument why we did not find any relevant clinical improvement, is that the margin of improvement is very small in both living donors and their recipients. Pre-, peri- and postoperative care has improved drastically in the last decade, for instance in reducing the impact of the surgery in the donor by favouring less invasive procedures, and in tailoring the immunosuppressive therapy in the recipients (Lentine et al., 2019; Poggio et al., 2021; Hariharan et al., 2021), reducing the burden of the procedures.

When looking back on our study design, given the results, we mainly question the bioavailability of SFN in our study participants. Participants in the intervention received a daily dose of 8 mg SFN, and given the duration of the diet of 5 days, a total expected dose of 40 mg was achieved. Regarding bioavailability, we did not measure the plasma level of total SFN or the urine level of SFN metabolites, which could have given us information to what extent the ingested SFN was bioactive and therefore potentially influencing the postoperative inflammatory response. Given the absence or only small positive effect of SFN found in this trial, we hypothesize that the bioavailability was not high enough. In hindsight, we could have given a higher cumulative dose of SFN. Earlier studies investigated (higher) dosages of SFN and its bioavailability, noting that there is significant difference in metabolism of sulforaphane (Zhang and Callaway, 2002; Oliviero et al., 2018; Gasper et al., 2005), possibly explaining the difference and/or absence of effect in our trial. During the design of our study, we established that in order to consume 90 mg of SFN, participants would have needed to eat 100 g of cress, which amounts to 10 portions, which we deemed highly unfeasible. Unpublished data, taken into consideration when designing our study, found that the dosage 90 mg did not significantly differ from the lower dosage, and therefore we opted for the dosage of 9 mg.

Limitations of this study should be acknowledged. First, the study sample size might have limited the statistical power to detect small but potentially meaningful effects, both in the inflammatory response and the clinical outcomes. Second, as stated before, there was no way to be sure if the dosage of 8 mg of SFN was sufficient for each donor in the intervention group. Additionally, the study primarily focused on short-term outcomes of the donor and recipient, long-term (>5 years) follow-up may provide further insights into the effects of the intervention. Moreover, the study relied on self-reported data for patient-reported outcomes, which could introduce bias or measurement error. However, it is important to note that donors were blinded to the

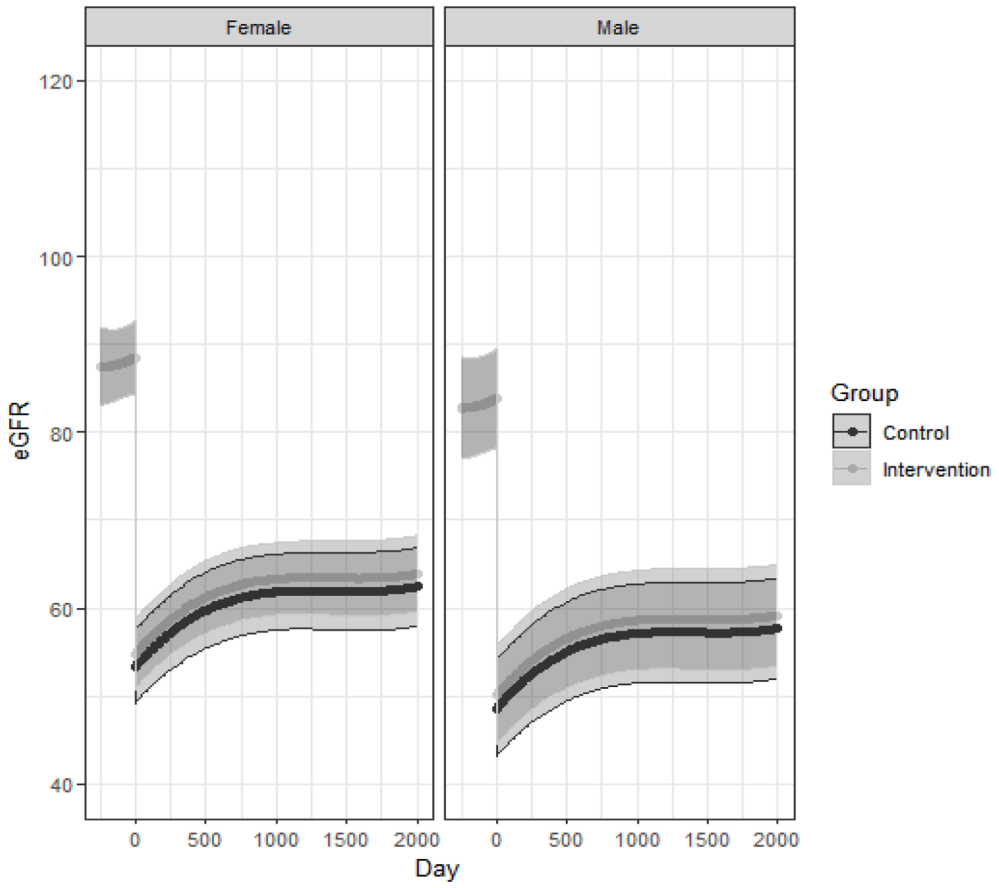

Fig. 9. Effect Plot eGFR Donor. Legend: This Effect Plot was constructed by the LMM-Analysis, and shows the expected change of eGFR over time, and the difference between the control and intervention groups. Both groups have a projected mean and an associated 95% prediction interval. X-Axis: Time (days), Y-Axis: eGFR via CKD-EPI (ml/min).

intervention with minimal chance of information bias in this setting.

5. Conclusion

In conclusion, a preoperative low-dose SFN-enriched diet did not consistently impact pre- and postoperative serum cytokine and adhesion molecule concentrations, or the expression of NRF2 target genes. Patient-reported outcomes showed no significant effect of the intervention on physical functioning and general health, except for a potential but non-significant improvement in physical functioning. Postoperative recovery of kidney function in both donors and recipients did not consistently show significant effects of the SFN-diet, although there were some small and non-consistent findings suggesting a possible positive impact of the intervention. It is important to note that these findings are small and therefore not clinically significant. Overall, the study suggests that the low-dose SFN-enriched diet did not have substantial and consistent effects on the measured outcomes in kidney donors and recipients. Further research into this specific SFN-enriched diet in this setting does not seem warranted given this study, but other avenues could be explored (Fahey and Kensler, 2021). Additionally, we raise the question of limited dosage and bioavailability. Given our findings, follow-up studies should take care to supplement a higher dosage and validate the bioavailable compound after ingestion. Given this study, we expect that restricting or increasing one ingredient/component does not significantly affect the inflammatory response and thereby improve overall recovery in this population. Other preconditioning methods such as caloric restriction or short-term fasting may hold more promise (Jongbloed et al., 2016; Jongbloed et al., 2020; van den Boogaard et al., 2021; Longo and Mattson, 2014; López-Otín et al., 2016; Lee et al., 2021; Reeves et al., 2013; van Ginhoven et al., 2011; Zhan et al., 2018), and could potentially be combined for a synergistic effect.

6. Trial sponsors

Erasmus University Medical Center, Rotterdam, The Netherlands. Koppert Cress BV, Monster, The Netherlands. Research and writing of this manuscript were indirectly supported by CoppertCress, but they had no part in formulating the hypothesis, construction of the study design or conduction of the trial.

7. Authors' contributions

The following authors have been extensively involved in forming the hypothesis, designing the study, and designing and formulating the study intervention: R.W.F. de Bruin, L.S.S. Ooms, T. Terkivatan and J.N. M IJzermans. During study runtime, the inclusion of potential study participants was performed by L.S.S. Ooms, J.W. Selten and E. van Straalen. G. Ambagtsheer performed all laboratory experiments. The collection, management, analysis, and interpretation of data was performed by C.A.J. Oudmaijer. The writing of the report and decision to submit was made by J.N.M. IJzermans, R.W.F. de Bruin and C.A.J. Oudmaijer. Thorough review of this article was performed by L.S.S. Ooms, J.W. Selten, E. van Straalen, G. Ambagtsheer and T. Terkivatan.

8. Declaration of generative AI in scientific writing

During the preparation of this work the authors used Trinka in order

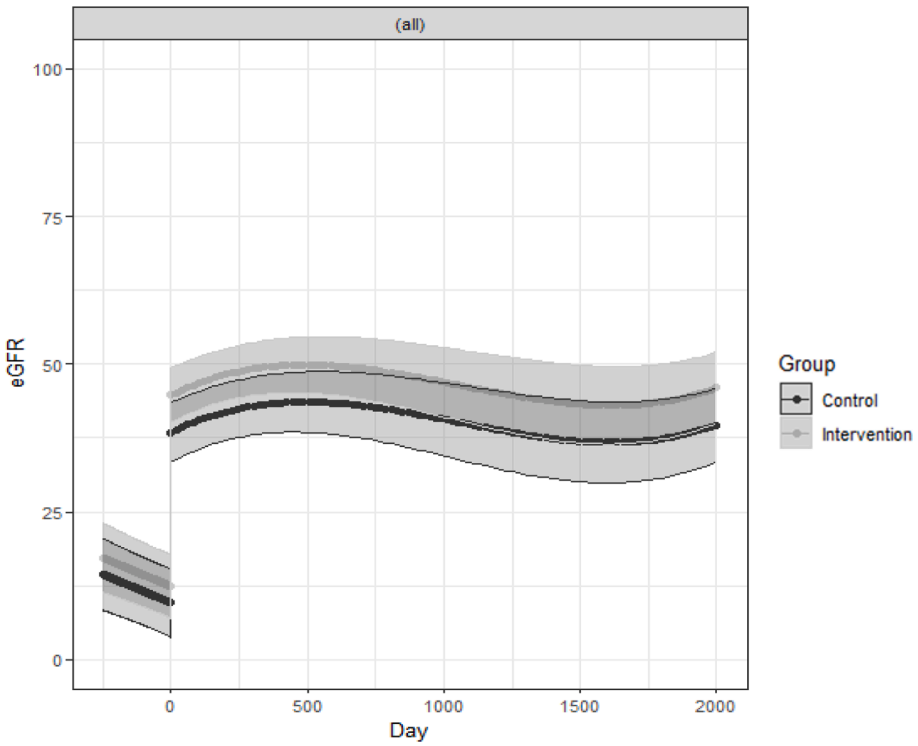

Fig. 10. Effect Plot eGFR Recipient. Legend: This Effect Plot was constructed by the LMM-Analysis, and shows the expected change of eGFR over time, and the difference between the control and intervention recipients. Both groups have a projected mean and an associated 95% prediction interval. X-Axis: Time (days), Y-Axis: eGFR via CKD-EPI (ml/min).

to check the grammar, spelling and wirting. After using this AI tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

9. Ethics Statement

Ethical approval for the involvement of human subjects in this study was granted by the medical ethical committee of Erasmus MC. This committee approved the study protocol, patient information files, consent procedures, and other study-related documents and procedures. The trial has been registered under medical ethical assessment numbers MEC-2015-452 and NL53890.078.15.

We have attached a copy of this approval.

10. Funding statement

Research and writing of this manuscript were indirectly supported by Koppert Cress BV, Monster, the Netherlands. They had no part in formulating the hypothesis, design of the study, conduction of the trial data analysis or writing the manuscript.

CRediT authorship contribution statement

C.A.J. Oudmaijer: Data curation, Formal analysis, Methodology, Project administration, Software, Writing – original draft, Writing – review & editing. **R.W.F. de Bruin:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing. **L.S.S. Ooms:** . **J.W. Selten:** Investigation, Project administration. **E. van Straalen:** . **G. Ambagtsheer:** Formal analysis, Investigation, Writing – review & editing. **T. Terkivatan:** Conceptualization, Project administration, Supervision, Writing – review & editing. **J.N.M. Ijzermans:** Conceptualization, Project administration, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2024.106161.

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