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Protocol for a pilot single-centre, parallel-arm, randomised controlled trial of dietary inulin to improve gut health in solid organ transplantation: the DIGEST Study

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ABSTRACT

Introduction

Kidney transplantation remains the best treatment for end-stage kidney disease, however the requirement for indefinite immunosuppression increases the risk of cardiovascular disease, cancer, and infection, leading to a reduction in long-term patient and graft survival. The gut microbiome is a critical determinant of health and modulates host immunity and metabolism through a number of recognised pathways, including through the production of immunomodulatory short-chain fatty acids (SCFA). Dietary supplementation with non-digestible fibre can augment the microbial production of SCFA and lead to favourable immune and metabolic outcomes, although this has yet to be shown in human kidney transplant recipients.

Methods and analysis

Dietary inulin for gut health in solid-organ transplantation (DIGEST) is a single-centre, unblinded, pilot parallel arm randomised controlled trial designed to assess the feasibility and adherence of dietary inulin, a naturally occurring dietary fibre, in the early post-transplant period in kidney transplant recipients. Participants will be

randomised at day 28 post-transplant to a four-week period of dietary inulin (10g-20g/day) in addition to standard care, or standard care alone, and followed-up until week 12 post-transplant.

The primary outcomes of the study are: i) the feasibility of participant recruitment, randomisation, and retention; ii) adherence to the intervention (inulin); and iii) the tolerability of inulin determined by changes in gastrointestinal symptoms as scored on the Gastrointestinal Symptom Rating Scale (GSRS).

Secondary outcomes include: (1) glycaemic variability determined by continuous glucose monitoring (CGM); (2) abundance of SCFA producing microbiota, as determined by 16s rRNA sequencing of the faecal metagenome; (3) serum SCFA concentrations; (4) peripheral blood immune cell populations; (5) recipient inflammatory and metabolic profiles; and (6) the incidence of biopsy proven acute rejection and kidney function determined by estimated glomerular filtration rate (eGFR).

Ethics and dissemination

All study visits, clinical, and laboratory assessments will be integrated into usual post-transplant care, creating no additional health-care encounters or procedures. The risks associated with this study are minor. Inulin has been shown to be well tolerated across a variety of cohorts, with the occurrence of short-term adverse gastrointestinal symptoms self-limiting. However, with gastrointestinal adverse events common following kidney transplantation, the tolerability of inulin in this cohort remains unknown. The results of DIGEST will be published in peer-reviewed journals and presented at academic conferences. This study has been approved by the Sydney Local Health District's Ethics Committee (Royal Prince Alfred Hospital Zone).

Trial registration

Australia and New Zealand Clinical Trials Registry Number:

ACTRN12620000623998

Strengths and limitations of this study

- DIGEST is a pioneering trial in the emerging field of microbiota research, and is the first step to investigate whether prebiotics can favourably alter the gut microbiome in kidney transplant recipients and ultimately improve health outcomes.
- DIGEST employs a pragmatic approach to trial design with all study visits, clinical, and laboratory assessments conducted in parallel with usual post-transplant care, creating no additional healthcare encounters for participants.
- The use of continuous glucose monitoring will provide unique insights into the characteristics and evolution of dysglycaemia in the early post-transplant period.
- Patient compliance and comfort with stool collection are enhanced through the use of an all-in-one system for easy self-collection, stabilisation, and preservation of stool samples by participants in their home environment.

- In this open-label study, a glass of water without an added placebo is used as the comparator arm due to limitations in obtaining a placebo with inert actions on both the microbiome and glycaemia.

INTRODUCTION

Kidney transplantation is the treatment of choice for the majority of patients with end-stage kidney disease. Compared to enduring dialysis, kidney transplantation delivers superior survival and quality of life at a reduced financial cost.[1-3] However, in the absence of long-term immunosuppression, immune mediated rejection of the transplant invariably occurs.[4] Modern immunosuppressive regimes, whilst effective at reducing the development of acute rejection, do so at considerable expense to both the patient and the allograft. For the majority of kidney transplant recipients, eventual loss of a kidney transplant occurs through either death with a functioning graft, or through chronic alloimmune mediated injury.[5] The most common causes of death post-transplant (cardiovascular disease, cancer, and infection) are all promoted by immunosuppression.[6] Furthermore, the mainstay of current treatment regimes, calcineurin inhibitors, paradoxically contribute to premature graft loss through the development of chronic allograft nephropathy.[7 8] Innovative strategies are hence needed to reduce the burden of current immunosuppression and maintain the balance between therapeutic efficacy and drug toxicity.

The gut microbiome is a critical determinant of human health and modulates host immunity and metabolism through a number of recognised pathways.[9] In transplantation, the gut microbiome offers a novel pathway to modify maladaptive immune and metabolic responses driven by alloantigen exposure and immunosuppression. Microbiota derived metabolites, and short-chain fatty acids (SCFA) in particular, are emerging as key mediators of the gut microbiomes influence over organ systems, including the immune system.[10] Formed by the fermentation of non-digestible dietary fibre by specific colonic bacteria, SCFAs may exert local effects on gut mucosa or act systemically, following systemic absorption, via engagement of specific G-protein coupled receptors or by inhibiting histone deacetylases (HDACs) and altering epigenetic regulation of gene expression.[11 12] In animal studies, SCFAs or their precursors (dietary-fibre) have been shown to attenuate kidney ischaemia-reperfusion injury,[13] and retard the development of allograft rejection,[14] as we have recently shown in a kidney allograft model.[15] Furthermore, post-transplant hyperglycaemia caused by the commonly used immunosuppressive agent, tacrolimus, has been linked to a reduction in intestinal

SCFA producing bacteria, with SCFA supplementation sufficient to prevent or correct the dysglycaemic response.[16]

Kidney transplantation results in considerable disruption to the gut microbiome, although the complexity and high interindividual variation in microbial communities of recipients has thus far impaired the ability of small studies to identify universal microbial markers predictive of post-transplant events.[17-19] However, the abundance of SCFA producing bacteria in recipients of allogeneic haematopoietic stem cell transplants has been associated with resistance against respiratory viral infection,[20] suggesting a further role for SCFA in promoting protective immunity post-transplant. Taken together, a growing body of evidence suggests that dietary supplementation to increase the presence of SCFA producing bacteria may not only promote transplant tolerance, but protect against infective complications and reduce one of the greatest contributors to post-transplant morbidity and mortality in dysglycaemia.

Inulin is a naturally occurring non-digestible dietary fibre composed of fructo-oligosaccharide (FOS) polymers.[21] In cohorts of healthy and overweight adults, dietary inulin has been shown to effectively promote the growth of SCFA producing

bacteria and increase serum SCFA concentrations,[22-25] leading to an improvement in metabolic parameters, including insulin resistance, and glycaemic control.[26-28] Dose regimens of inulin and inulin-type fructans in interventional studies have varied widely,[26] however a fixed dose of 20g/day is sufficient to generate changes in gut microbiota composition whilst limiting untoward gastrointestinal effects.[29]

Whilst dietary pre-biotics have been shown to promote the growth of SCFA producing bacteria in healthy and dysglycaemic cohorts, no studies have examined whether the gut microbiota are similarly amenable to intervention in the post-transplant period. Given the high incidence of dysglycaemia and glucose variability in the post-transplant cohort,[30 31] the potential to derive a meaningful advantage from even small improvements in glucose metabolism is significant.

This pilot, randomised controlled trial will assess the feasibility and adherence to dietary inulin supplementation, and explore the effect of inulin on gut bacterial communities and glycaemic variability in the early post-transplant period. This trial aims to provide data to inform the feasibility, design, and viability of larger clinical

trials to assess the efficacy of dietary prebiotics in improving outcomes for kidney transplant recipients.

METHODS AND ANALYSIS

Study design

The Dietary Inulin for Gut Health in Solid-organ Transplantation (DIGEST) study is a single-centre, unblinded, parallel-arm randomised controlled trial of 40 adult kidney transplant recipients. The trial will screen patients for enrolment from post-transplant day 14 and randomise participants in a 1:1 ratio at post-transplant day 28 to a four-week period of either a) supplementation with dietary inulin in addition to standard post-transplant care; or b) standard post-transplant care alone. Study participants will be followed until post-transplant week 12. An outline of the study design is illustrated in Figure 1.

Study aim and hypothesis

The primary aims of DIGEST are to determine the feasibility of recruitment, randomisation, and retention; and the adherence to and tolerance of inulin supplementation in the early post-transplant period. The secondary outcomes seek to evaluate the effect of inulin supplementation on the relative abundance of SCFA producing gut bacteria, glycaemic variability, metabolic profiles, immune cell populations, and graft outcomes. These exploratory outcomes will assess data collection and outcome measures, and will be used to inform the design of a definitive trial.

We hypothesise that dietary prebiotic inulin is feasible, safe, and well tolerated in the early post-transplant period. In addition, we seek to show that inulin will favourably enhance the relative abundance of SCFA producing gut bacteria and reduce the range of glycaemic variability post-transplant.

Target population and eligibility criteria

DIGEST will enrol adult (18 years or older) recipients of kidney transplants performed at Royal Prince Alfred Hospital, a quarternary-care hospital in Sydney, Australia from 16 June 2020 until the recruitment target is met.

Inclusion criteria

The inclusion and exclusion criteria are shown in Table 1. DIGEST will recruit both diabetic and non-diabetic kidney transplant recipients (KTRs) from deceased or living donors who are willing and able to provide written informed consent and are able to comply with all trial and follow-up requirements for the duration of the study.

Inclusion Criteria
Recipients of a kidney transplant from a living or deceased donor
Individuals aged ≥ 18 years old who are able to give informed consent, and a willingness to participate and comply with the study requirements
Patients who receive a kidney from an ABO blood group incompatible donor, or as part of the Paired Kidney Exchange Program
Exclusion Criteria
Individuals diagnosed with significant gastro-intestinal diseases e.g. inflammatory bowel disease, coeliac disease
Patients who receive anti-thymocyte globulin (ATG) as induction or treatment for rejection
Acute rejection in the first 4 weeks post-transplant
Delayed Graft Function requiring dialysis persisting beyond week 2 post-transplant
Presence of gastrointestinal tract output stoma

Current enrolment in another intervention or investigational drug trial
Recipients of multi-organ transplants
Known food intolerance, allergy, or sensitivity to inulin or dietary fibre.
Inability or unwillingness of individual or legal guardian to give written informed consent

Table 1. Inclusions and exclusion criteria

Exclusion criteria

The complete exclusion criteria are shown in Table 1. Conditions with the potential to confound the interpretation of the intervention (inulin) on outcome measures will be excluded. This includes; 1) KTRs with significant gastrointestinal disorders (such as coeliac disease or inflammatory bowel disease) or a prior colonic resection; 2) KTRs at higher immune risk who receive T-cell depleting agents and those with acute rejection who are subsequently exposed to greater levels of immunosuppression, and; 3) KTRs with delayed graft function requiring dialysis beyond post-transplant day 14.

Recruitment and randomisation

Potential participants will be identified for inclusion in the study by clinical staff or a member of the research team, either at the time of their initial hospital stay following kidney transplantation, or during their regular attendance at the acute transplant

clinic following hospital discharge. All potentially eligible patients will be screened for enrolment, provided with a study information sheet, and if agreeable, receive information regarding the study from a research team member. Prior to performing any study specific procedure, a signed consent form will be obtained for each subject. For subjects without capacity to consent, a signed consent form will be obtained from their legal guardian. A study investigator will conduct the informed consent discussion to ensure that the patient and/or legal representative comprehends the information provided, and that all queries or concerns are addressed. Consent will be voluntary and free from coercion.

Randomisation will occur through computer-generated permuted block randomisation using a block size of 6, with participants assigned in a 1:1 ratio to either a) supplementation with dietary inulin dissolved in 200mls water twice daily in addition to standard post-transplant care; or b) 200ml water twice daily in addition to standard post-transplant care. Randomisation will be stratified by sex and diabetic status at the time of study enrolment to maintain the balance of confounders between the two study arms. The randomisation schedule will be generated by the "blockrand"[32] package for R, and administered through a central web-based

randomisation module contained within the REDCap data management platform,[33] ensuring concealment of the allocation sequence from investigators. DIGEST is an open-label trial, with participants and investigators unblinded to study-arm allocation once randomisation has occurred. Following completion of all data collection, the study-arms will be relabelled with non-identifying terms by a third-party to blind the data analysts to treatment allocation.

Intervention period

The intervention period of the DIGEST trial will run for four weeks, commencing at day 28 post-transplant.

Intervention group

Participants randomised to the intervention arm, will receive inulin 10 grams each morning for the first 7 days, increasing to 10 grams morning and night for the remaining 21 days of the intervention period. A run-in period of inulin 10 grams once-daily followed by dose escalation to 10 grams twice-daily after one week has been utilised to minimise the development of early dose-related unacceptable adverse gastrointestinal effects. Inulin will be provided in powdered form and contained within

individual 10-gram sachets. The contents of each sachet will be self-administered by the study participant by dissolving in approximately 200mls of water prior to consuming. The study supplement will be acquired from MYPROTEIN in its commercially available form.

Inulin is a naturally occurring fructo-oligosaccharide (FOS) composed of heterogenous polymers of 2-60 d-fructose units with each ending in a terminal glucosyl unit. It may be produced synthetically through enzymatic action on sucrose or extracted from a natural source i.e. chicory root. It is available as a retail food additive or dietary supplement in a powdered form and is readily soluble in water with a neutral unflavoured taste.

Participants randomised to the intervention group will continue with standard post-transplant care as documented below.

Monitoring

Subjects who experience persistent mild adverse effects (bowel discomfort, bloating, flatulence) will be instructed to reduce the dose to 10 grams/day. Subjects who are unable to continue with the supplement will discontinue the study intervention and

continue with all study visits and assessments. All protocol deviations and adverse events will be recorded.

Standard care group

During the intervention period, participants in the standard care group are asked to consume one glass of water (approximately 200mls) each morning for the first 7 days, increasing to morning and night for the following 21 days.

Participants in both arms of the study will receive standard-post transplant care as per the local practice guidelines. This includes standard immunosuppression for the prevention of immune mediated rejection (calcineurin inhibitor, mycophenolate, and glucocorticoids), and supportive treatments to manage medical risks post-transplant such as anti-microbial prophylaxis for pneumocystis jiroveci, cytomegalovirus, and candida, in addition to anti-hypertensive and hypoglycaemic medications at the direction of their transplant physician. All patients receive medication education through a pharmacist, and nutritional assessment and advice by a renal dietician

either during their inpatient hospital stay or as part of their regular follow-up in the acute transplant clinic.

Post-randomisation visits and assessment

DIGEST employs a pragmatic approach to trial design with all study visits, clinical, and laboratory assessments conducted in parallel with usual post-transplant care. For study participants, this creates no additional hospital encounters, exposes participants to no further medical procedures (including venepuncture), reduces the time commitment required for participation, and will aid in the follow-up and retention of enrolled participants.

A schematic diagram of the schedule of enrolment, interventions, and assessments is provided by Figure 3.

Primary outcomes

The DIGEST study has been developed to collect and report outcome data in accordance with the CONSORT extension to randomised pilot and feasibility trials (Figure 1).[34]

Feasibility of recruitment and retention

The feasibility of patient recruitment to the DIGEST trial will be determined by the percentage of patients screened who proceed to randomisation. The feasibility of retaining patients throughout the study will be assessed by the percentage of randomised patients who complete all study visits, and the feasibility of implementing the study protocol by the percentage of randomised patients who complete all outcome measures. The feasibility of recruitment, retention, and implementation will be determined at the completion of the final study visit by the last enrolled participant.

Adherence and tolerability of Inulin

The adherence to inulin will be determined by the number of subjects randomised to the intervention arm who consume at least 10 grams of inulin each day for ≥80% of the intervention period. To enhance the validity of data, multiple methods will be used to assess intervention inherence. Following allocation, participants will be questioned on the frequency of missed, skipped, or omitted doses, and the number of consumed and unopened inulin sachets will be counted at each study visit. Participants will be reminded at each study visit of the importance of adherence to the study intervention and questioned for the development of adverse events. All reasons for non-adherence will be documented.

The tolerability of inulin supplementation determined by the development of adverse gastrointestinal effects will be assessed using the Gastrointestinal Symptom Rating Scale (GSRS). The GSRS is a validated, self-administered questionnaire which assesses the severity of gastrointestinal symptoms using a 7-point Likert scale across five domains: indigestion, diarrhoea, constipation, abdominal pain, and reflux. It has previously been validated in the renal transplant population.[35 36] The GSRS will

be administered to participants in both trial arms just prior to, and one week following the commencement of the intervention period, at the end of the intervention period, and at post-transplant week 12. Patients who require either dose reduction or cessation of inulin due to adverse gastrointestinal effects will be recorded.

Safety

All adverse events will be recorded using an adaptation of the National Institute of Health's Common Terminology Criteria for Adverse Events by a study team member. All study participants will be screened for the occurrence of adverse events at each study visit by a member of the research team, and during their regular assessment by a transplant physician in the Acute Transplant Clinic. All serious adverse events (SAEs) will be reported to the study sponsor within 24 hours of the study team becoming aware of the event.

Secondary Outcomes

Secondary outcomes for the DIGEST study are exploratory in nature and will be performed to examine the feasibility of data collection and biospecimen analysis, and to inform the viability of the study protocol for a definitive trial.

Continuous glucose monitoring

Glycaemic control and variability will be measured through continuous glucose monitoring (CGM) conducted over two 14-day periods using a Libre Freestyle sensor. CGM sensors will be placed on all study participants at one week prior to the commencement, and one week prior to the cessation of the intervention period. Data generated from CGM will be analysed and reported in line with current guidelines for CGM reporting.[37]

Clinical and laboratory measurements

Study participants will have clinical and laboratory outcomes assessed at three time points during the study: (i) prior to the commencement of the intervention period; (ii)

within the last week of the intervention period; and (iii) at the final study visit occurring at post-transplant week 12. These measures include; weight and body mass index, blood pressure, serum creatinine, lipid profile, fasting glucose and insulin levels, and blood short-chain fatty acid levels. Plasma and peripheral blood mononuclear cells (PBMCs) isolated from whole blood will be aliquoted and stored for biochemical analysis and immunophenotyping by cytometry, respectively. Non-diabetic patients will be asked to undergo a 75-gram oral glucose tolerance test following week 10 post-transplant to assess for the presence of impaired glucose tolerance (IGT) or the occult occurrence of new onset diabetes after transplant (NODAT). Indication and 12-week protocol biopsies of the kidney transplant will be performed at the discretion of the participant's treating clinicians.

Faecal microbiota

16s rRNA sequencing of the faecal metagenome isolated from stool samples collected at the above timepoints will be used to determine the diversity, composition and relative abundance of bacteria within the gut microbiome. Faecal specimens are collected using an all-in-one system (OMNIgene GUT OM-200, DNA Genotek,

CANADA) for easy self-collection by the participant in their home environment and point-of-collection stabilisation of faecal DNA at room-temperature, until it can be returned to a study team member. An assessment of habitual diet will be determined by 4-day food diaries recorded at the time of stool sample collection and analysed via FoodWorks.

Patient and public involvement

Whilst the impetus for this pilot study is derived principally from our pre-clinical research, informal discussions with kidney transplant recipients revealed significant interest in the relationship between diet, kidney health, and metabolic disease following transplantation, and a willingness to participate in research. Prior to finalisation of the study protocol, we consulted three recent kidney transplant recipients regarding the design, implementation, and data collection methods for this trial. Their feedback resulted in modifications to the Participant Information sheet and adaptation of the study visits and data collection tools to more adequately respond to

the needs of patients in the early post-transplant period, and to reduce the burden of time required to participate in the research.

Sample size calculation

This pilot study aims to determine the tolerability and feasibility of inulin supplementation in the early post-transplant period, with exploratory outcomes that may provide initial data for primary outcome measures in order to calculate sample size for a larger trial.

The use of metrics derived from continuous glucose monitoring (CGM) remains a novel outcome measure. We have previously demonstrated in a cohort of 28 renal transplant recipients significant variations in glucose homeostasis with the use of continuous glucose monitoring, identifying a role for CGM in predicting patients who will go on to develop post-transplant diabetes.[30] Interventional studies assessing the impact of dietary fibre on glucose metabolism have not reported CGM outcomes, whilst commonly reported measures such as HbA1c have decreased diagnostic utility in the early post-transplant period.[38]

Using reported data of available glycaemic metrics, a metanalysis examining the metabolic benefits of dietary prebiotics in non-transplant cohorts found that dietary prebiotics (fibre) improved post-prandial glucose levels with a standardised mean difference of -0.79 mmol/L (95% CI -1.41, -0.12).[27] For a main trial designed to detect a standardised effect size of 0.8 at 90% power and two-sided 5% significance, pilot trial sample sizes of 10 per treatment arm have been recommended.[39] We therefore aim to recruit 20 subjects per treatment arm, allowing for both drop-out and a margin of error in effect size.

Data analysis plan

The intention-to-treat (ITT) principle for all outcomes will be applied to the final analyses with patients assessed according to their trial arm allocation; however, we will also report per-protocol results.[40] The per-protocol analyses will exclude patients from the treatment arm who failed to adhere or tolerate inulin supplementation, or consumed <80% of the prescribed supplement.

The following analyses will be performed for the primary outcome: 1) recruitment and retention of trial participants, will be reported as the percentage of patients screened who proceed to randomisation, and the percentage of randomised patients who completed all study procedures and follow-up requirements, respectively; 2) adherence to inulin will be reported as the percentage of participants randomised to the intervention arm who adhere to inulin supplementation for the full prescribed period ; and 3) the tolerability of inulin determined by longitudinal changes in GSRS scores analysed using a generalised linear mixed model with study arm, time, sex, age, immunosuppression type, antibiotic use in the prior week (yes or no), and the interaction between study arm and time as the main fixed effects. The random effect of subject will be used to account for the repeated interdependent observations from each study subject. Data missing at random will be handled in a mixed model using maximum likelihood estimates. If > 10% of primary outcome data is determined to be missing not at random, a best-worst and worst-best case sensitivity analyses will be performed.

Baseline characteristics of participants and recorded outputs will be expressed as means ± standard deviations for normally distributed data or the median ±

interquartile range for non-normally distributed data, and as frequencies for categorical variables. Differences in continuous variables between the study arms will be assessed using students' t-test for normally distributed data, or by the non-parametric Wilcoxon signed rank test for non-normally distributed data. Categorical variables will be compared using the Chi squared and Fisher's exact test as appropriate. Exploratory outcome measures will be analysed by univariate and multivariate methods with adjustments for multiple comparisons where required. A pre-defined subgroup analysis by diabetic status will be performed for the exploratory outcomes. A 2-sided significance level of 5% will be used for all analyses.

Data management and access

A Research Data Management Plan (RDMP) has been created and approved under local ethics governance. All study data is entered directly onto study specific electronic data capture forms coordinated through a secure web-based data management tool called REDCap.[33] The stored data is maintained on protected

servers with the Sydney Local Health District data centre, ensuring provision of back-up, privacy, and confidentiality requirements. Access to the secure REDCap DIGEST database is available only to research team members. REDCap requires individual user authentication, ensures data logging, and utilises Secure Sockets Layer (SSL) encryption, allowing data integrity and monitoring to be upheld as per the RDMP. All biobanked laboratory specimens are labelled with a unique coded identifier to maintain participant confidentiality.

ETHICS AND DISSEMINATION

The safety and tolerability of inulin has been widely studied across a number of cohorts where it has been shown to be safe and well tolerated in doses exceeding 20g/day. [41-43] The possible benefits to participants include any potential benefit derived from the study intervention, additionally participants with prior or new onset diabetes after transplant may derive benefit from the periods of continuous glucose monitoring with the reduced requirement for burdensome finger-prick testing of capillary blood glucose.

The results of DIGEST are anticipated to be published in peer reviewed scientific journals and presented at academic meetings. We anticipate the findings will be of interest across a broad field of transplant clinicians, nephrologists, dieticians, and consumers. Identifiable data will not be publicly released and deidentified data may be made available upon reasonable request from interested investigators, with the exception of faecal metagenome sequencing reads which will be uploaded to the European Nucleotide Archive (EMBL-EBI). Trial data will be held by the Sydney Local Health District and the University of Sydney for a minimum period of 15 years.

DISCUSSION

Assessing the feasibility, adherence, and tolerance of dietary fibre supplementation by kidney transplant recipients is the first step in determining whether prebiotic fibre may have a role as an adjunctive therapy to improve outcomes for KTRs. The information derived from this pilot study will provide estimates of the eligibility, recruitment, and retention rates that will inform the design, size, and feasibility of future studies. Larger scale trials will be required to more conclusively determine

whether dietary supplementation can alter the gut microbiome post-transplant, and ultimately improve transplant outcomes.

Modifying the gut microbiota by dietary supplementation is a novel and promising strategy to promote health outcomes that has garnered much public interest.[44 45] Indeed, consultation with our own patients suggests a willingness to engage in this area of research. Taken with the knowledge that dietary supplement use is common in Australia, with 47% of women and 34% of men consuming supplements regularly,[46] we believe there will be significant interest in this study by potential participants, in addition to the scientific community.

Prebiotic dietary fibres are a key nutritional source for SCFA-producing gut bacteria (e.g. bifidobacteria and lactobacillus) and promote their selective growth.[22 23] As the prototypical prebiotic, inulin has been commonly used as an investigational product and has established a reputable body of literature demonstrating both safety and efficacy. [42 43 47 48] Together with inulin's wide availability and low cost, it forms an ideal investigational supplement for use in the post-transplant period. However, with

gastrointestinal adverse events common in KTRs,[49] the adherence and tolerability of inulin supplementation in this cohort remains unknown and requires investigation.

The early post-transplant period presents numerous challenges for kidney transplant recipients that must be acknowledged. New medications with frequent dosing changes, adaptation to new healthcare settings, intercurrent illness, and an exposure to an excess of information may all reduce KTRs willingness to participate in clinical trials.[50] In this regard, there are a number of factors that we believe will enhance interest and participation in this trial. The DIGEST trial creates no additional healthcare encounters for participants, which is of critical importance during this period of increased risk with COVID-19. Furthermore, the short follow-up period in this pilot trial is intended to enhance participation during the "acute phase" of post-transplant care, prior to a patient's discharge from the primary transplant centre, often to distant locations.

DIGEST is not without limitations. The unique physical and metabolic properties of inulin (soluble, non-digestible, fermentable fibre) preclude the attainment of a suitable placebo that will maintain an inert effect on both participant glycaemia and

the gut microbiome, whilst preserving participant and investigator blinding to treatment allocation. Previous controls in inulin trials have frequently included maltodextrins[51 52], which have a high glycaemic index, or non-fermentable microcrystalline cellulose[53 54], which is insoluble in water. Furthermore, we acknowledge that commencing a dietary supplement to establish a favourable microbiome prior to transplant would increase the likelihood of attaining a microbiota derived benefit. However, this would limit participation to a smaller cohort of living-donor transplant recipients or render wait-listed patients to long-term dietary supplementation.

Ultimately, the DIGEST pilot study will provide important information in a novel area of transplant medicine and inform the direction of future interventional trials.

Author contributions

The study was conceived and designed by JS, LM, HW, and SC. JS wrote the first draft of the DIGEST protocol and prepared the manuscript. YL, TY, LA, DG, KW, LM, HW and SC participated in critical review of the study protocol, and revisions to the

manuscript. All authors contributed significant intellectual content to the protocol and approved the final manuscript.

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Competing interests

The authors have no competing interest to declare.

Figure legends

Figure 1. Study flowchart for the DIGEST pilot trial, adapted from the CONSORT extension to randomised pilot and feasibility trials.

Figure 2. Schematic diagram demonstrating the schedule of enrolment, intervention, assessments, and visits for study participants. CGM, continuous glucose monitoring. GSRS, gastrointestinal symptoms rating scale. 1 For non-diabetic participants. 2 At the discretion of the participants treating team.

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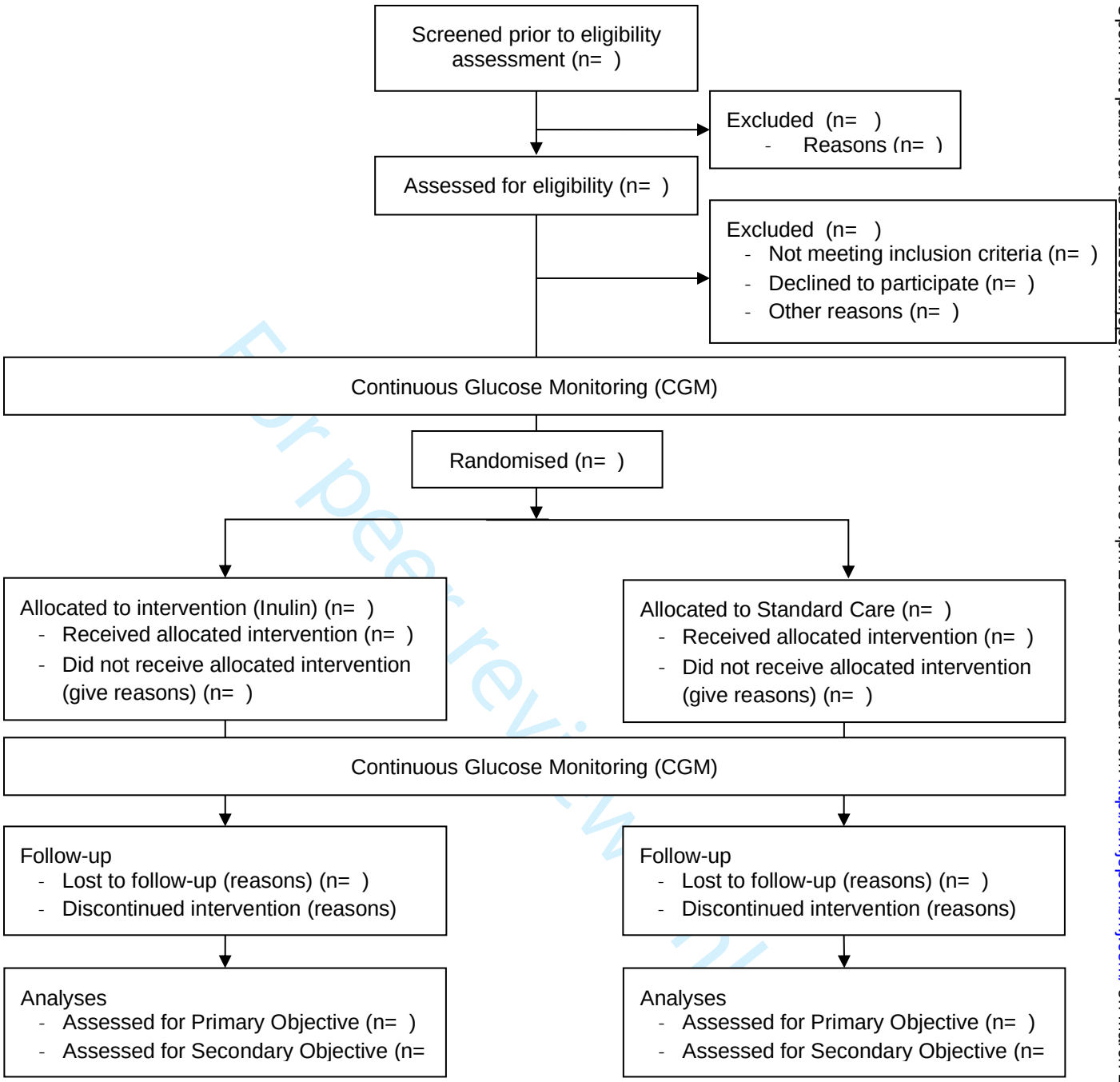

Figure 1. Study flowchart for the DIGEST pilot trial, adapted from the CONSORT extension to randomised pilot and feasibility trials.

	STUDY PERIOD								
	Enrolment		Allocation	Post-allocation					Close-out
TIMEPOINT (time in weeks)	*- 2*	*-1*	*0*	*1*	*2*	*3*	*4*	*5*	*8 ± 1*
ENROLMENT:									
Eligibility screen	X								
Informed consent	X								
Allocation			X						
INTERVENTIONS:									
Inulin + Standard Care				◆—	—	—	—◆		
Standard Care				◆—	—	—	—◆		
ASSESSMENTS:									
Demographics	X								
Transplant characteristics	X								
CGM		◆—	—	—◆			◆—	—◆	
Height	X								
Weight	X	X	X	X			X	X	X
Blood pressure	X	X	X	X			X	X	X
Current Medications	X	X	X	X			X	X	X
GSRS			X	X			X		X
Haematological and biochemical tests			X				X		X
Stool collection			X				X		X
4-day food diary			X				X		X
75g OGTT[1]									X
Protocol biopsy[2]									X
Adherence assessment				X	X	X	X		
Adverse event assessment			X	X	X	X	X	X	X

Figure 2. Schematic diagram demonstrating the schedule of enrolment, intervention, assessments, and visits for study participants. CGM, continuous glucose monitoring. GSRS, gastrointestinal symptoms rating scale. [1] For non-diabetic participants. [2] At the discretion of the participants treating team.

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	#3	Date and version identifier	NA
Funding	#4	Sources and types of financial, material, and other support	22
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	22

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	22
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	NA
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	6
Objectives	#7	Specific objectives or hypotheses	6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will	9

		perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	11
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	12
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	14
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	13
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 2
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	17
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	10
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	10
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	18
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	18
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	18
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	18

Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	18
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	NA
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	14
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	3
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	19

Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	20
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	20
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	NA
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	20
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

BMJ Open

Protocol for a pilot single-centre, parallel-arm, randomised controlled trial of dietary inulin to improve gut health in solid organ transplantation: the DIGEST Study

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Secondary Subject Heading:	Diabetes and endocrinology, Pharmacology and therapeutics, Research methods
Keywords:	Renal transplantation < NEPHROLOGY, Transplant medicine < INTERNAL MEDICINE, IMMUNOLOGY, General diabetes < DIABETES & ENDOCRINOLOGY

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Protocol for a pilot single-centre, parallel-arm, randomised controlled trial of dietary inulin to improve gut health in solid organ transplantation: the DIGEST Study

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ABSTRACT

Introduction

Kidney transplantation remains the best treatment for end-stage kidney disease, however the requirement for indefinite immunosuppression increases the risk of cardiovascular disease, cancer, and infection, leading to a reduction in long-term patient and graft survival. The gut microbiome is a critical determinant of health and modulates host immunity and metabolism through a number of recognised pathways, including through the production of immunomodulatory short-chain fatty acids (SCFA). Dietary supplementation with non-digestible fibre can augment the microbial production of SCFA and lead to favourable immune and metabolic outcomes, although this has yet to be shown in human kidney transplant recipients.

Methods and analysis

Dietary inulin for gut health in solid-organ transplantation (DIGEST) is a single-centre, unblinded, pilot parallel arm randomised controlled trial designed to assess the feasibility and adherence of dietary inulin, a naturally occurring dietary fibre, in the early post-transplant period in kidney transplant recipients. Participants will be

randomised at day 28 post-transplant to a four-week period of dietary inulin (10g-20g/day) in addition to standard care, or standard care alone, and followed-up until week 12 post-transplant.

The primary outcomes of the study are: i) the feasibility of participant recruitment, randomisation, and retention; ii) adherence to the intervention (inulin); and iii) the tolerability of inulin determined by changes in gastrointestinal symptoms as scored on the Gastrointestinal Symptom Rating Scale (GSRS).

Secondary outcomes include: (1) glycaemic variability determined by continuous glucose monitoring (CGM); (2) abundance of SCFA producing microbiota, as determined by 16s rRNA sequencing of the faecal metagenome; (3) serum SCFA concentrations; (4) peripheral blood immune cell populations; (5) recipient inflammatory and metabolic profiles; and (6) the incidence of biopsy proven acute rejection and kidney function determined by estimated glomerular filtration rate (eGFR).

Ethics and dissemination

All study visits, clinical, and laboratory assessments will be integrated into usual post-transplant care, creating no additional health-care encounters or procedures. The risks associated with this study are minor. Inulin has been shown to be well tolerated across a variety of cohorts, with the occurrence of short-term adverse gastrointestinal symptoms self-limiting. However, with gastrointestinal adverse events common following kidney transplantation, the tolerability of inulin in this cohort remains unknown. The results of DIGEST will be published in peer-reviewed journals and presented at academic conferences. This study has been approved by the Sydney Local Health District's Ethics Committee (Royal Prince Alfred Hospital Zone).

Trial registration

Australia and New Zealand Clinical Trials Registry Number:

ACTRN12620000623998

Strengths and limitations of this study

- DIGEST uses a randomised controlled design to assess the pleotropic effects of dietary inulin in the early period following kidney transplantation.
- DIGEST employs a pragmatic approach to trial design with all study visits, clinical, and laboratory assessments conducted in parallel with usual post-transplant care, creating no additional healthcare encounters for participants.
- 4-day food diaries will capture dietary intake at multiple timepoints to assess for intra- and interindividual variation in habitual diet and fibre intake.
- In this open-label study, a glass of water without an added placebo is used as the comparator arm due to limitations in obtaining a placebo with inert actions on both the microbiome and glycaemia.
- As a single-centre study, the results may not be generalisable to all populations.

INTRODUCTION

Kidney transplantation is the treatment of choice for the majority of patients with end-stage kidney disease. Compared to enduring dialysis, kidney transplantation delivers superior survival and quality of life at a reduced financial cost.[1-3] However, in the absence of long-term immunosuppression, immune mediated rejection of the transplant invariably occurs.[4] Modern immunosuppressive regimes, whilst effective at reducing the development of acute rejection, do so at considerable expense to both the patient and the allograft. For the majority of kidney transplant recipients, eventual loss of a kidney transplant occurs through either death with a functioning graft, or through chronic alloimmune mediated injury.[5] The most common causes of death post-transplant (cardiovascular disease, cancer, and infection) are all promoted by immunosuppression.[6] Furthermore, the mainstay of current treatment regimes, calcineurin inhibitors, paradoxically contribute to premature graft loss through the development of chronic allograft nephropathy.[7 8] Innovative strategies are hence needed to reduce the burden of current immunosuppression and maintain the balance between therapeutic efficacy and drug toxicity.

The gut microbiome is a critical determinant of human health and modulates host immunity and metabolism through a number of recognised pathways.[9] In transplantation, the gut microbiome offers a novel pathway to modify maladaptive immune and metabolic responses driven by alloantigen exposure and immunosuppression. Microbiota derived metabolites, and short-chain fatty acids (SCFA) in particular, are emerging as key mediators of the gut microbiomes influence over organ systems, including the immune system.[10] Formed by the fermentation of non-digestible dietary fibre by specific colonic bacteria, SCFAs may exert local effects on gut mucosa or act systemically, following systemic absorption, via engagement of specific G-protein coupled receptors or by inhibiting histone deacetylases (HDACs) and altering epigenetic regulation of gene expression.[11 12] In animal studies, SCFAs or their precursors (dietary-fibre) have been shown to attenuate kidney ischaemia-reperfusion injury,[13] and retard the development of allograft rejection,[14] as we have recently shown in a kidney allograft model.[15] Furthermore, post-transplant hyperglycaemia caused by the commonly used immunosuppressive agent, tacrolimus, has been linked to a reduction in intestinal

SCFA producing bacteria, with SCFA supplementation sufficient to prevent or correct the dysglycaemic response.[16]

Kidney transplantation results in considerable disruption to the gut microbiome, although the complexity and high interindividual variation in microbial communities of recipients has thus far impaired the ability of small studies to identify universal microbial markers predictive of post-transplant events.[17-19] However, the abundance of SCFA producing bacteria in recipients of allogeneic haematopoietic stem cell transplants has been associated with resistance against respiratory viral infection,[20] suggesting a further role for SCFA in promoting protective immunity post-transplant. Taken together, a growing body of evidence suggests that dietary supplementation to increase the presence of SCFA producing bacteria may not only promote transplant tolerance, but protect against infective complications and reduce one of the greatest contributors to post-transplant morbidity and mortality in dysglycaemia.

Inulin is a naturally occurring non-digestible dietary fibre composed of fructo-oligosaccharide (FOS) polymers.[21] In cohorts of healthy and overweight adults, dietary inulin has been shown to effectively promote the growth of SCFA producing

bacteria and increase serum SCFA concentrations,[22-25] leading to an improvement in metabolic parameters, including insulin resistance, and glycaemic control.[26-28] Dose regimens of inulin and inulin-type fructans in interventional studies have varied widely,[26] however a fixed dose of 20g/day is sufficient to generate changes in gut microbiota composition whilst limiting untoward gastrointestinal effects.[29]

Whilst dietary pre-biotics have been shown to promote the growth of SCFA producing bacteria in healthy and dysglycaemic cohorts, no studies have examined whether the gut microbiota are similarly amenable to intervention in the post-transplant period. Given the high incidence of dysglycaemia and glucose variability in the post-transplant cohort,[30 31] the potential to derive a meaningful advantage from even small improvements in glucose metabolism is significant.

This pilot, randomised controlled trial will assess the feasibility and adherence to dietary inulin supplementation, and explore the effect of inulin on gut bacterial communities and glycaemic variability in the early post-transplant period. This trial aims to provide data to inform the feasibility, design, and viability of larger clinical

trials to assess the efficacy of dietary prebiotics in improving outcomes for kidney transplant recipients.

METHODS AND ANALYSIS

Study design

The Dietary Inulin for Gut Health in Solid-organ Transplantation (DIGEST) study is a single-centre, unblinded, parallel-arm randomised controlled trial of 40 adult kidney transplant recipients. The trial will screen patients for enrolment from post-transplant day 14 and randomise participants in a 1:1 ratio at post-transplant day 28 to a four-week period of either a) supplementation with dietary inulin in addition to standard post-transplant care; or b) standard post-transplant care alone. Study participants will be followed until post-transplant week 12. An outline of the study design is illustrated in Figure 1.

Study aim and hypothesis

The primary aims of DIGEST are to determine the feasibility of recruitment, randomisation, and retention; and the adherence to and tolerance of inulin supplementation in the early post-transplant period. The secondary outcomes seek to evaluate the effect of inulin supplementation on the relative abundance of SCFA producing gut bacteria, glycaemic variability, metabolic profiles, immune cell populations, and graft outcomes. These exploratory outcomes will assess data collection and outcome measures, and will be used to inform the design of a definitive trial.

We hypothesise that dietary prebiotic inulin is feasible, safe, and well tolerated in the early post-transplant period. In addition, we seek to show that inulin will favourably enhance the relative abundance of SCFA producing gut bacteria and reduce the range of glycaemic variability post-transplant.

Target population and eligibility criteria

DIGEST will enrol adult (18 years or older) recipients of kidney transplants performed at Royal Prince Alfred Hospital, a quarternary-care hospital in Sydney, Australia from 16 June 2020 until the recruitment target is met.

Inclusion criteria

The inclusion and exclusion criteria are shown in Table 1. DIGEST will recruit both diabetic and non-diabetic kidney transplant recipients (KTRs) from deceased or living donors who are willing and able to provide written informed consent and are able to comply with all trial and follow-up requirements for the duration of the study.

Inclusion Criteria
Recipients of a kidney transplant from a living or deceased donor
Individuals aged ≥ 18 years old who are able to give informed consent, and a willingness to participate and comply with the study requirements
Patients who receive a kidney from an ABO blood group incompatible donor, or as part of the Paired Kidney Exchange Program
Exclusion Criteria
Individuals diagnosed with significant gastro-intestinal diseases e.g. inflammatory bowel disease, coeliac disease
Patients who receive anti-thymocyte globulin (ATG) as induction or treatment for rejection
Acute rejection in the first 4 weeks post-transplant
Delayed Graft Function requiring dialysis persisting beyond week 2 post-transplant
Presence of gastrointestinal tract output stoma

Current enrolment in another intervention or investigational drug trial
Recipients of multi-organ transplants
Known food intolerance, allergy, or sensitivity to inulin or dietary fibre.
Inability or unwillingness of individual or legal guardian to give written informed consent

Table 1. Inclusions and exclusion criteria

Exclusion criteria

The complete exclusion criteria are shown in Table 1. Conditions with the potential to confound the interpretation of the intervention (inulin) on outcome measures will be excluded. This includes; 1) KTRs with significant gastrointestinal disorders (such as coeliac disease or inflammatory bowel disease) or a prior colonic resection; 2) KTRs at higher immune risk who receive T-cell depleting agents and those with acute rejection who are subsequently exposed to greater levels of immunosuppression, and; 3) KTRs with delayed graft function requiring dialysis beyond post-transplant day 14.

Recruitment and randomisation

Potential participants will be identified for inclusion in the study by clinical staff or a member of the research team, either at the time of their initial hospital stay following kidney transplantation, or during their regular attendance at the acute transplant

clinic following hospital discharge. All potentially eligible patients will be screened for enrolment, provided with a study information sheet, and if agreeable, receive information regarding the study from a research team member. Prior to performing any study specific procedure, a signed consent form will be obtained for each subject. For subjects without capacity to consent, a signed consent form will be obtained from their legal guardian. A study investigator will conduct the informed consent discussion to ensure that the patient and/or legal representative comprehends the information provided, and that all queries or concerns are addressed. Consent will be voluntary and free from coercion.

Randomisation will occur through computer-generated permuted block randomisation using a block size of 6, with participants assigned in a 1:1 ratio to either a) supplementation with dietary inulin dissolved in 200mls water twice daily in addition to standard post-transplant care; or b) 200ml water twice daily in addition to standard post-transplant care. Randomisation will be stratified by sex and diabetic status at the time of study enrolment to maintain the balance of confounders between the two study arms. The randomisation schedule will be generated by the "blockrand"[32] package for R, and administered through a central web-based

randomisation module contained within the REDCap data management platform,[33] ensuring concealment of the allocation sequence from investigators. DIGEST is an open-label trial, with participants and investigators unblinded to study-arm allocation once randomisation has occurred. Following completion of all data collection, the study-arms will be relabelled with non-identifying terms by a third-party to blind the data analysts to treatment allocation.

Intervention period

The intervention period of the DIGEST trial will run for four weeks, commencing at day 28 post-transplant.

Intervention group

Participants randomised to the intervention arm, will receive inulin 10 grams each morning for the first 7 days, increasing to 10 grams morning and night for the remaining 21 days of the intervention period. A run-in period of inulin 10 grams once-daily followed by dose escalation to 10 grams twice-daily after one week has been utilised to minimise the development of early dose-related unacceptable adverse gastrointestinal effects. Inulin will be provided in powdered form and contained within

individual 10-gram sachets. The contents of each sachet will be self-administered by the study participant by dissolving in approximately 200mls of water prior to consuming. The study supplement will be acquired from MYPROTEIN in its commercially available form.

Inulin is a naturally occurring fructo-oligosaccharide (FOS) composed of heterogenous polymers of 2-60 d-fructose units with each ending in a terminal glucosyl unit. It may be produced synthetically through enzymatic action on sucrose or extracted from a natural source i.e. chicory root. It is available as a retail food additive or dietary supplement in a powdered form and is readily soluble in water with a neutral unflavoured taste.

Participants randomised to the intervention group will continue with standard post-transplant care as documented below.

Monitoring

Subjects who experience persistent mild adverse effects (bowel discomfort, bloating, flatulence) will be instructed to reduce the dose to 10 grams/day. Subjects who are unable to continue with the supplement will discontinue the study intervention and

continue with all study visits and assessments. All protocol deviations and adverse events will be recorded.

Standard care group

During the intervention period, participants in the standard care group are asked to consume one glass of water (approximately 200mls) each morning for the first 7 days, increasing to morning and night for the following 21 days.

Participants in both arms of the study will receive standard-post transplant care as per the local practice guidelines. This includes standard immunosuppression for the prevention of immune mediated rejection (calcineurin inhibitor, mycophenolate, and glucocorticoids), and supportive treatments to manage medical risks post-transplant such as anti-microbial prophylaxis for pneumocystis jiroveci, cytomegalovirus, and candida, in addition to anti-hypertensive and hypoglycaemic medications at the direction of their transplant physician. All patients receive medication education through a pharmacist, and nutritional assessment and advice by a renal dietician either during their inpatient hospital stay or as part of their regular follow-up in the

acute transplant clinic. Enroled participants will be instructed to continue with their normal diet and to not increase the consumption of fibre rich foods.

Post-randomisation visits and assessment

DIGEST employs a pragmatic approach to trial design with all study visits, clinical, and laboratory assessments conducted in parallel with usual post-transplant care. For study participants, this creates no additional hospital encounters, exposes participants to no further medical procedures (including venepuncture), reduces the time commitment required for participation, and will aid in the follow-up and retention of enrolled participants.

A schematic diagram of the schedule of enrolment, interventions, and assessments is provided by Figure 2.

Primary outcomes

The DIGEST study has been developed to collect and report outcome data in accordance with the CONSORT extension to randomised pilot and feasibility trials (Figure 1).[34]

Feasibility of recruitment and retention

The feasibility of patient recruitment to the DIGEST trial will be determined by the percentage of patients screened who proceed to randomisation. The feasibility of retaining patients throughout the study will be assessed by the percentage of randomised patients who complete all study visits, and the feasibility of implementing the study protocol by the percentage of randomised patients who complete all outcome measures. The feasibility of recruitment, retention, and implementation will be determined at the completion of the final study visit by the last enrolled participant.

Adherence and tolerability of Inulin

The adherence to inulin will be determined by the number of subjects randomised to the intervention arm who consume at least 10 grams of inulin each day for ≥80% of the intervention period. To enhance the validity of data, multiple methods will be used to assess intervention inherence. Following allocation, participants will be questioned on the frequency of missed, skipped, or omitted doses, and the number of consumed and unopened inulin sachets will be counted at each study visit. Participants will be reminded at each study visit of the importance of adherence to the study intervention and questioned for the development of adverse events. All reasons for non-adherence will be documented.

The tolerability of inulin supplementation determined by the development of adverse gastrointestinal effects will be assessed using the Gastrointestinal Symptom Rating Scale (GSRS). The GSRS is a validated, self-administered questionnaire which assesses the severity of gastrointestinal symptoms using a 7-point Likert scale across five domains: indigestion, diarrhoea, constipation, abdominal pain, and reflux. It has previously been validated in the renal transplant population.[35 36] The GSRS will

be administered to participants in both trial arms just prior to, and one week following the commencement of the intervention period, at the end of the intervention period, and at post-transplant week 12. Patients who require either dose reduction or cessation of inulin due to adverse gastrointestinal effects will be recorded.

Safety

All adverse events will be recorded using an adaptation of the National Institute of Health's Common Terminology Criteria for Adverse Events by a study team member. All study participants will be screened for the occurrence of adverse events at each study visit by a member of the research team, and during their regular assessment by a transplant physician in the Acute Transplant Clinic. All serious adverse events (SAEs) will be reported to the study sponsor within 24 hours of the study team becoming aware of the event.

Secondary Outcomes

Secondary outcomes for the DIGEST study are exploratory in nature and will be performed to examine the feasibility of data collection and biospecimen analysis, and to inform the viability of the study protocol for a definitive trial.

Continuous glucose monitoring

Glycaemic control and variability will be measured through continuous glucose monitoring (CGM) conducted over two 14-day periods using a Libre Freestyle sensor. CGM sensors will be placed on all study participants at one week prior to the commencement, and one week prior to the cessation of the intervention period. Data generated from CGM will be analysed and reported in line with current guidelines for CGM reporting.[37]

Clinical and laboratory measurements

Study participants will have clinical and laboratory outcomes assessed at three time points during the study: (i) prior to the commencement of the intervention period; (ii)

within the last week of the intervention period; and (iii) at the final study visit occurring at post-transplant week 12. These measures include; weight and body mass index, blood pressure, serum creatinine, lipid profile, fasting glucose and insulin levels, and blood short-chain fatty acid levels. Plasma and peripheral blood mononuclear cells (PBMCs) isolated from whole blood will be aliquoted and stored for biochemical analysis and immunophenotyping by cytometry, respectively. Non-diabetic patients will be asked to undergo a 75-gram oral glucose tolerance test following week 10 post-transplant to assess for the presence of impaired glucose tolerance (IGT) or the occult occurrence of new onset diabetes after transplant (NODAT). Indication and 12-week protocol biopsies of the kidney transplant will be performed at the discretion of the participant's treating clinicians.

Faecal microbiota

16s rRNA sequencing of the faecal metagenome isolated from stool samples collected at the above timepoints will be used to determine the diversity, composition and relative abundance of bacteria within the gut microbiome. Faecal specimens are collected using an all-in-one system (OMNIgene GUT OM-200, DNA Genotek,

CANADA) for easy self-collection by the participant in their home environment and point-of-collection stabilisation of faecal DNA at room-temperature, until it can be returned to a study team member. An assessment of habitual diet will be determined by 4-day food diaries recorded at the time of stool sample collection and analysed via FoodWorks.

Patient and public involvement

Whilst the impetus for this pilot study is derived principally from our pre-clinical research, informal discussions with kidney transplant recipients revealed significant interest in the relationship between diet, kidney health, and metabolic disease following transplantation, and a willingness to participate in research. Prior to finalisation of the study protocol, we consulted three recent kidney transplant recipients regarding the design, implementation, and data collection methods for this trial. Their feedback resulted in modifications to the Participant Information sheet and adaptation of the study visits and data collection tools to more adequately respond to

the needs of patients in the early post-transplant period, and to reduce the burden of time required to participate in the research.

Sample size calculation

This pilot study aims to determine the tolerability and feasibility of inulin supplementation in the early post-transplant period, with exploratory outcomes that may provide initial data for primary outcome measures in order to calculate sample size for a larger trial.

The use of metrics derived from continuous glucose monitoring (CGM) remains a novel outcome measure. We have previously demonstrated in a cohort of 28 renal transplant recipients significant variations in glucose homeostasis with the use of continuous glucose monitoring, identifying a role for CGM in predicting patients who will go on to develop post-transplant diabetes.[30] Interventional studies assessing the impact of dietary fibre on glucose metabolism have not reported CGM outcomes, whilst commonly reported measures such as HbA1c have decreased diagnostic utility in the early post-transplant period.[38]

Using reported data of available glycaemic metrics, a metanalysis examining the metabolic benefits of dietary prebiotics in non-transplant cohorts found that dietary prebiotics (fibre) improved post-prandial glucose levels with a standardised mean difference of -0.79 mmol/L (95% CI -1.41, -0.12).[27] For a main trial designed to detect a standardised effect size of 0.8 at 90% power and two-sided 5% significance, pilot trial sample sizes of 10 per treatment arm have been recommended.[39] We therefore aim to recruit 20 subjects per treatment arm, allowing for both drop-out and a margin of error in effect size.

Data analysis plan

The intention-to-treat (ITT) principle for all outcomes will be applied to the final analyses with patients assessed according to their trial arm allocation; however, we will also report per-protocol results.[40] The per-protocol analyses will exclude patients from the treatment arm who failed to adhere or tolerate inulin supplementation, or consumed <80% of the prescribed supplement.

The following analyses will be performed for the primary outcome: 1) recruitment and retention of trial participants, will be reported as the percentage of patients screened who proceed to randomisation, and the percentage of randomised patients who completed all study procedures and follow-up requirements, respectively; 2) adherence to inulin will be reported as the percentage of participants randomised to the intervention arm who adhere to inulin supplementation for the full prescribed period ; and 3) the tolerability of inulin determined by longitudinal changes in GSRS scores analysed using a generalised linear mixed model with study arm, time, sex, age, immunosuppression type, antibiotic use in the prior week (yes or no), and the interaction between study arm and time as the main fixed effects. The random effect of subject will be used to account for the repeated interdependent observations from each study subject. Data missing at random will be handled in a mixed model using maximum likelihood estimates. If > 10% of primary outcome data is determined to be missing not at random, a best-worst and worst-best case sensitivity analyses will be performed.

Baseline characteristics of participants and recorded outputs will be expressed as means ± standard deviations for normally distributed data or the median ±

interquartile range for non-normally distributed data, and as frequencies for categorical variables. Differences in continuous variables between the study arms will be assessed using students' t-test for normally distributed data, or by the non-parametric Wilcoxon signed rank test for non-normally distributed data. Categorical variables will be compared using the Chi squared and Fisher's exact test as appropriate. Exploratory outcome measures will be analysed by univariate and multivariate methods with adjustments for multiple comparisons where required. A pre-defined subgroup analysis by diabetic status will be performed for the exploratory outcomes. A 2-sided significance level of 5% will be used for all analyses.

Data management and access

A Research Data Management Plan (RDMP) has been created and approved under local ethics governance. All study data is entered directly onto study specific electronic data capture forms coordinated through a secure web-based data management tool called REDCap.[33] The stored data is maintained on protected

servers with the Sydney Local Health District data centre, ensuring provision of back-up, privacy, and confidentiality requirements. Access to the secure REDCap DIGEST database is available only to research team members. REDCap requires individual user authentication, ensures data logging, and utilises Secure Sockets Layer (SSL) encryption, allowing data integrity and monitoring to be upheld as per the RDMP. All biobanked laboratory specimens are labelled with a unique coded identifier to maintain participant confidentiality.

ETHICS AND DISSEMINATION

The safety and tolerability of inulin has been widely studied across a number of cohorts where it has been shown to be safe and well tolerated in doses exceeding 20g/day. [41-43] The possible benefits to participants include any potential benefit derived from the study intervention, additionally participants with prior or new onset diabetes after transplant may derive benefit from the periods of continuous glucose monitoring with the reduced requirement for burdensome finger-prick testing of capillary blood glucose.

The results of DIGEST are anticipated to be published in peer reviewed scientific journals and presented at academic meetings. We anticipate the findings will be of interest across a broad field of transplant clinicians, nephrologists, dieticians, and consumers. Identifiable data will not be publicly released and deidentified data may be made available upon reasonable request from interested investigators, with the exception of faecal metagenome sequencing reads which will be uploaded to the European Nucleotide Archive (EMBL-EBI). Trial data will be held by the Sydney Local Health District and the University of Sydney for a minimum period of 15 years.

This study has been approved by the Sydney Local Health District's Ethics Committee (Royal Prince Alfred Hospital Zone) under project ID 2019/PID14472.

DISCUSSION

Assessing the feasibility, adherence, and tolerance of dietary fibre supplementation by kidney transplant recipients is the first step in determining whether prebiotic fibre may have a role as an adjunctive therapy to improve outcomes for KTRs. The information derived from this pilot study will provide estimates of the eligibility, recruitment, and retention rates that will inform the design, size, and feasibility of

future studies. Larger scale trials will be required to more conclusively determine whether dietary supplementation can alter the gut microbiome post-transplant, and ultimately improve transplant outcomes.

Modifying the gut microbiota by dietary supplementation is a novel and promising strategy to promote health outcomes that has garnered much public interest.[44 45] Indeed, consultation with our own patients suggests a willingness to engage in this area of research. Taken with the knowledge that dietary supplement use is common in Australia, with 47% of women and 34% of men consuming supplements regularly,[46] we believe there will be significant interest in this study by potential participants, in addition to the scientific community.

Prebiotic dietary fibres are a key nutritional source for SCFA-producing gut bacteria (e.g. bifidobacteria and lactobacillus) and promote their selective growth.[22 23] As the prototypical prebiotic, inulin has been commonly used as an investigational product and has established a reputable body of literature demonstrating both safety and efficacy. [42 43 47 48] Together with inulin's wide availability and low cost, it forms an ideal investigational supplement for use in the post-transplant period. However, with

gastrointestinal adverse events common in KTRs,[49] the adherence and tolerability of inulin supplementation in this cohort remains unknown and requires investigation.

The early post-transplant period presents numerous challenges for kidney transplant recipients that must be acknowledged. New medications with frequent dosing changes, adaptation to new healthcare settings, intercurrent illness, and an exposure to an excess of information may all reduce KTRs willingness to participate in clinical trials.[50] In this regard, there are a number of factors that we believe will enhance interest and participation in this trial. The DIGEST trial creates no additional healthcare encounters for participants, which is of critical importance during this period of increased risk with COVID-19. Furthermore, the short follow-up period in this pilot trial is intended to enhance participation during the "acute phase" of post-transplant care, prior to a patient's discharge from the primary transplant centre, often to distant locations.

DIGEST is not without limitations. The unique physical and metabolic properties of inulin (soluble, non-digestible, fermentable fibre) preclude the attainment of a suitable placebo that will maintain an inert effect on both participant glycaemia and

the gut microbiome, whilst preserving participant and investigator blinding to treatment allocation. Previous controls in inulin trials have frequently included maltodextrins[51 52], which have a high glycaemic index, or non-fermentable microcrystalline cellulose[53 54], which is insoluble in water. Furthermore, we acknowledge that commencing a dietary supplement to establish a favourable microbiome prior to transplant would increase the likelihood of attaining a microbiota derived benefit. However, this would limit participation to a smaller cohort of living-donor transplant recipients or render wait-listed patients to long-term dietary supplementation.

Ultimately, the DIGEST pilot study will provide important information in a novel area of transplant medicine and inform the direction of future interventional trials.

Author contributions

The study was conceived and designed by JS, LM, HW, and SC. JS wrote the first draft of the DIGEST protocol and prepared the manuscript. YL, TY, LA, DG, KW, LM, HW and SC participated in critical review of the study protocol, and revisions to the

manuscript. All authors contributed significant intellectual content to the protocol and approved the final manuscript.

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Competing interests

The authors have no competing interest to declare.

Figure legends

Figure 1. Study flowchart for the DIGEST pilot trial, adapted from the CONSORT extension to randomised pilot and feasibility trials.

Figure 2. Schematic diagram demonstrating the schedule of enrolment, intervention, assessments, and visits for study participants. CGM, continuous glucose monitoring. GSRS, gastrointestinal symptoms rating scale. 1 For non-diabetic participants. 2 At the discretion of the participants treating team.

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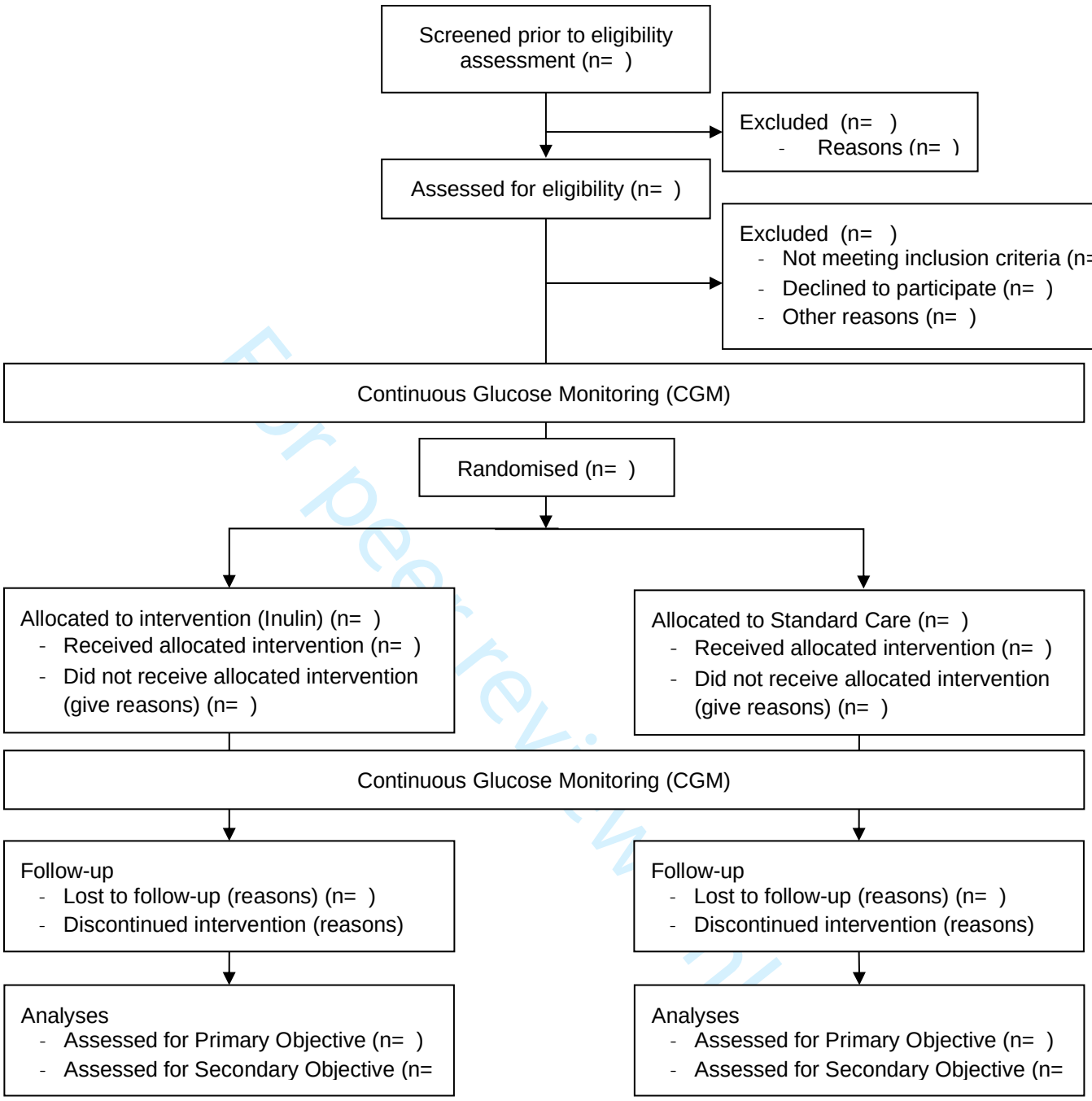

Figure 1. Study flowchart for the DIGEST pilot trial, adapted from the CONSORT extension to randomised pilot and feasibility trials.

	STUDY PERIOD								
	Enrolment		Allocation	Post-allocation					Close-out
TIMEPOINT (time in weeks)	*- 2*	*-1*	*0*	*1*	*2*	*3*	*4*	*5*	*8 ± 1*
ENROLMENT:									
Eligibility screen	X								
Informed consent	X								
Allocation			X						
INTERVENTIONS:									
Inulin + Standard Care				←	—	—	→		
Standard Care				←	—	—	→		
ASSESSMENTS:									
Demographics	X								
Transplant characteristics	X								
CGM		←	—	→			←	→	
Height	X								
Weight	X	X	X	X			X	X	X
Blood pressure	X	X	X	X			X	X	X
Current Medications	X	X	X	X			X	X	X
GSRS			X	X			X		X
Haematological and biochemical tests			X				X		X
Stool collection			X				X		X
4-day food diary			X				X		X
75g OGTT[1]									X
Protocol biopsy[2]									X
Adherence assessment				X	X	X	X		
Adverse event assessment			X	X	X	X	X	X	X

Figure 2. Schematic diagram demonstrating the schedule of enrolment, intervention, assessments, and visits for study participants. CGM, continuous glucose monitoring. GSRS, gastrointestinal symptoms rating scale. [1] For non-diabetic participants. [2] At the discretion of the participants treating team.

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	#3	Date and version identifier	NA
Funding	#4	Sources and types of financial, material, and other support	22
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	22

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	22
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	NA
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	6
Objectives	#7	Specific objectives or hypotheses	6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will	9

		perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	11
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	12
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	14
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	13
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 2
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	17
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	10
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	10
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	18
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	18
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	18
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	18

Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	18
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	NA
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	14
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	3
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	19

Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	20
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	20
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	NA
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	20
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA