


BMJ Open Effect of daily consumption of probiotic yoghurt on albumin to creatinine ratio, eGFR and metabolic parameters in patients with type 2 diabetes with microalbuminuria: study protocol for a randomised controlled clinical trial

Seyed Mojtaba Ghoreishy ¹, Nooshin Shirzad,² Manouchehr Nakhjavani,² Alireza Esteghamati,² Kurosh Djafarian,¹ Ahmad Esmailzadeh^{3,4}

To cite: Ghoreishy SM, Shirzad N, Nakhjavani M, *et al.* Effect of daily consumption of probiotic yoghurt on albumin to creatinine ratio, eGFR and metabolic parameters in patients with type 2 diabetes with microalbuminuria: study protocol for a randomised controlled clinical trial. *BMJ Open* 2022;**12**:e056110. doi:10.1136/bmjopen-2021-056110

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2021-056110>).

Received 03 August 2021
Accepted 22 February 2022



© Author(s) (or their employer(s)) 2022. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

Correspondence to

Dr Ahmad Esmailzadeh;
a-esmailzadeh@tums.ac.ir

ABSTRACT

Introduction To alleviate clinical symptoms of diabetic nephropathy (DN), several dietary and non-dietary strategies have been suggested. Probiotic-enriched foods, through their effects on modulating microflora, might help these patients control the adverse effects. The current study will be done to examine the effects of probiotic yoghurt consumption on albumin to creatinine ratio, estimated glomerular filtration rate (eGFR) and metabolic parameters in patients with type 2 diabetes with nephropathy.

Methods and analysis Sixty patients with DN will be recruited in this study. After block matching for sex, body mass index and age, patients will be randomly assigned to receive 300 g/day probiotic yoghurt containing 10^6 CFU/g *Lactobacillus acidophilus* and *Bifidobacterium lactis* strains or 300 g/day plain yoghurt daily for 8 weeks. Weight, height and waist circumference will be measured at study baseline and after the intervention. Biochemical indicators including glycaemic measures (haemoglobin A1c (HbA1c), fasting blood sugar (FBS)), inflammatory markers (high sensitivity-C reactive protein), lipid profile (total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL)) and finally renal makers (creatinine, albumin to creatinine ratio, eGFR) will be assessed at study baseline and at the end of the trial.

Discussion Improving the condition of a person with DN is a serious clinical challenge. The use of probiotic supplements has been considered in these people, but the use of probiotic-enriched foods has received less attention.

Trial registration number Iranian Registry of Clinical Trials (www.irct.ir) (IRCT20201125049491N1).

BACKGROUND

Diabetic nephropathy (DN), a complication of diabetes, is one of the main causes of end-stage renal disease.^{1–5} It often results from an increase in base membrane thickness, mesangial cell proliferation, deposition of extracellular matrix proteins, and afferent

Strengths and limitations of this study

- In this study, patients will be block matched with each other in terms of several variables.
- This study will use probiotic strains available in the food market and will not focus on specific strains that may have different health effects.
- Compliance to the intervention will be assessed throughout the study.
- It would be better to quantify urinary protein excretion in a 24-hour urine sample; however, due to difficulties in the field, we would not be able to do this. Instead, we will examine the protein excretion in a spot urine sample in the early morning.

and efferent vascular hyalinosis, which eventually leads to glomerular fibrosis.^{2 4 6–8} In 2015, the number of people with diabetes was about 415 million worldwide, and this is anticipated to reach 642 million in 2040.^{1 3 9} It has been estimated that 40% of patients with diabetes eventually develop kidney disorders.¹⁰ Thus, it is important to consider the significant impact of this condition on individuals and society.^{1 3 9}

Finding a cheap and appropriate strategy to manage this condition is of great priority.^{1 11} Recent studies have shown that changes in intestinal microflora might play a role in the development of DN.¹² An increase in the number of harmful bacteria in the intestine has been associated with elevated uraemic toxins, such as NH₃, amines, indoles, thiols and phenols in individuals with gut microbiota dysbiosis.¹³ Some studies have administered probiotic supplements as a strategy for modulating gut microflora and reported favourable effects on renal function.^{14 15} For

instance, supplementation with probiotics in patients with nephropathy resulted in reduced serum concentration of uric acid and blood urea nitrogen (BUN).^{16–18} However, some studies failed to reach such a significant effect. Borges *et al* administered probiotic supplements for 12 weeks in patients with chronic kidney disease and did not show any significant effect on uraemic toxins and inflammatory markers.¹⁹ Some studies have shown that administration of probiotics in high doses in the form of dietary supplements might result in infection.^{20 21} In addition, prescription of these supplements would be associated with additional cost to patients and their family.^{16 22} This might result in discontinuing these supplements despite their probable beneficial effects.

Introduction of probiotic-enriched foods might be a good choice for taking benefits of these products among patients. Despite a number of studies on the impact of probiotic supplements on renal function, only a few studies have looked at the impact of probiotic-enriched foods. Abbasi *et al*, in a study on 44 patients with DN, indicated the favourable effects of consuming soy milk containing *Lactobacillus plantarum* A7 for 8 weeks on albumin to creatinine ratio, creatinine and glomerular filtration rate (GFR).¹ However, they did not report the effects on glycaemic factors. In addition, *L. plantarum* was rarely used in the available probiotic-enriched foods in the market; rather most available foods have used *Bifidobacterium lactis* and *L. acidophilus*. In a 6-week clinical trial on 64 patients with diabetes, consumption of probiotic yoghurt resulted in a significant reduction in haemoglobin A1c (HbA1c) and fasting blood glucose.²³ Given the limited evidence on this area, the present study aims to examine the effect of probiotic yoghurt consumption on renal, inflammatory and metabolic factors in patients with DN.

PATIENTS AND METHODS

Participants

The present study will be a parallel and double-blind controlled clinical trial among patients with DN (albumin to creatinine ratio 30–299 mg/g Cr, estimated GFR (eGFR) ≥ 30 mL/min/1.73 m²),²⁴ aged 20–70 years with a body mass index (BMI) < 40 kg/m². The study is designed to be done in the Diabetes Clinic of Imam Khomeini Hospital in Tehran, Iran. The intervention will start in June 2022 and will end in September 2022.

Inclusion criteria

In this study, we will recruit patients: (1) with DN with microalbuminuria (albumin to creatinine ratio 30–299 mg/g Cr, eGFR ≥ 30 mL/min/1.73 m²); (2) aged 20–70 years; (3) with BMI less than 40 kg/m²; (4) who have a fixed plan for medication use during the last 3 months.

Non-inclusion criteria

Patients will not be included if they (1) have a history of chronic diseases including inflammatory bowel disease

(IBD), liver disease, rheumatoid arthritis and other renal disorders except for DN; (2) have a history of smoking and alcohol use; (3) have a history of taking antibiotics and Non-steroidal anti-inflammatory drugs (NSAIDs) during the last month; (4) were taking omega 3, vitamins E and C supplements during the last 3 months; (5) were consuming probiotic supplements or foods containing probiotics during the last 3 months; (6) were pregnant or lactating women, individuals with lactose intolerance or those planning to get pregnant in the next 3 months.

Exclusion criteria

We will exclude the following patients: (1) those who get pregnant throughout the study; (2) those who have a weight change (± 3 kg) during the study; (3) those who change the dose of medications during the study; (4) those who are on a specific diet throughout the study.

All participants will read and sign the informed consent. This study has already been approved by the ethics committee of Tehran University of Medical Sciences (IR.TUMS.MEDICINE.REC.1399.1132).

Sample size calculation

Considering type I error of 5% and type II error of 20% and the study power of 80%, and urinary albumin as the key variable,²⁵ we computed the required sample size using the suggested formula for parallel clinical trials²⁶:

$$n = \frac{2 \left[\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta} \right)^2 \times S^2 \right]}{\Delta^2}$$

n=sample size in each group.

According to the above calculations, 22 people in each group (44 people in total) will be needed. Considering the possible drop-out of 20% and non-compliance of individuals, 30 people in each group (60 people in total) will enter this study.

Study design and intervention

A diagram of the study design is shown in figure 1. Individuals will be enrolled based on inclusion criteria. First, all study participants will pass a 2-week run-in period, during which three 24-hour food recalls (2 working days and 1 weekend day) will be used to collect dietary information. In addition, subjects will be requested to record their physical activity for 2 days (1 working day and 1 weekend day) during this period. Information about demographic characteristics, medical history, medication use as well as socioeconomic status will be collected in this period through the use of a questionnaire in a face-to-face interview. Then, at the end of the run-in period, anthropometric measures, glycaemic status, renal function, lipid profile, inflammatory markers and blood pressure will be evaluated. Participants will then be placed in blocks based on age (± 5 years), gender (male/female) and BMI (± 2 kg/m²). Each block will consist of two persons almost similar in terms of age and gender and BMI. Patients with a prespecified code will then be randomly assigned to the intervention and non-intervention groups by a third

CONSORT 2010 Flow Diagram

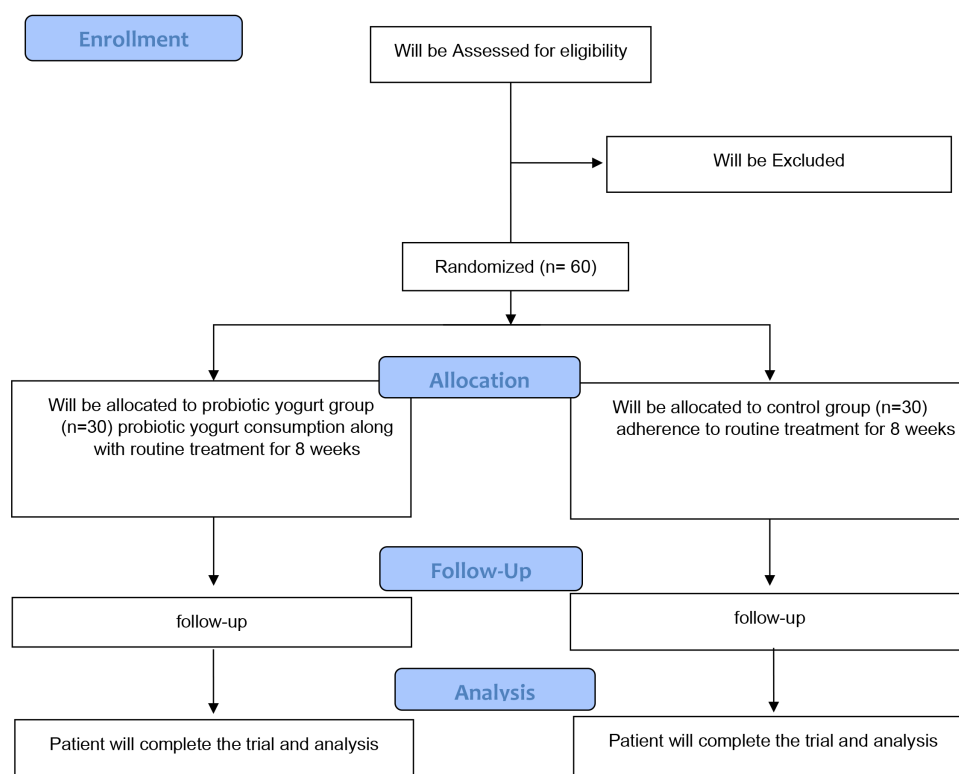


Figure 1 Diagram of the study design. CONSORT, Consolidated Standards of Reporting Trials.

person, who is not aware of the study aim. Study investigators, participants and the laboratory team will all be blind to the intervention. Patients in the intervention group (n=30) will consume 300g probiotic yoghurt per day (enriched with *B. lactis* and *L. acidophilus* strains at a dose of 10^6 CFU/g of live bacteria) for 8 weeks. Individuals in the non-intervention group (n=30) will take 300g plain yoghurt (without any added probiotics) per day for this time period. The yoghurt used in both groups will have the same flavour and appearance, and is hidden from researchers. The yoghurts that will be given to both groups will be packed in the same container to have the same flavour and appearance and there is no label on the container to identify the contents. Throughout the intervention, subjects will be asked to refrain from changing their daily diet and routine daily physical activity. Every 2 weeks, participants will get yoghurt packages. To examine compliance, a 24-hour dietary recall once every 2 weeks will be completed. All study outcomes will be examined again at the study end.

Adherence

Study subjects will be given a checklist to keep track of their daily probiotic or normal yoghurt intake. They will receive daily reminders on their mobile phones to promote compliance and prevent forgetting to consume probiotic or conventional yoghurt daily. In addition, each time yoghurt packages are delivered, patients will be

requested to return empty packages of consumed ones. Additional packages of conventional yoghurt will be offered to study participants for possible family members' use to ensure that the yoghurts are consumed by the study participants and not their family members.

Primary and secondary outcome measures

The primary study outcomes would be albumin to creatinine ratio. The study secondary outcome variables would be eGFR, glycaemic factors (HbA1c, fasting blood sugar (FBS)), inflammatory markers (high sensitivity-C reactive protein (hs-CRP)), lipid profile (total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL))

Dietary intake and physical activity assessment

To ensure the lack of change in dietary intakes of participants throughout the study, we will fill three 1-day dietary recalls every 2 weeks throughout the study. Dietary recall format is presented in the online supplemental file A. Due to the COVID-19 pandemic, dietary recalls will be completed through phone calls. Based on the examination of these dietary recalls, we will calculate the dietary intake of food and nutrients for study participants. Nutritionist IV will be used to perform this calculation.

Subjects will also be requested to record their physical activity for 2 days in order to examine the difference in activity between the two groups (one for a working day

and one for weekend). All participants will be educated on how to record their activities throughout the study before the start of the trial. Furthermore, patients in both groups will be asked not to change their physical activity throughout the trial. In order to analyse physical activity records, we will use Metabolic equivalent of task (MET)-hour/day values for each type of physical activity, based on published guidelines,²⁷ considering the time spent by each participant. The format of the physical activity record is shown in the online supplemental file B. All recalls and records would be reviewed immediately and possible problems will be resolved by interview.

Anthropometric measures

Data on anthropometric measurements will be collected at the study baseline and end of trial. Body weight will be measured in a fasting state, without shoes, with minimal clothing using digital scales (Seca, Hamburg, Germany) to the nearest 100 g. Standing height will be measured using a standard stadiometer without shoes with an accuracy of 0.5 cm. Waist circumference will be measured using a tape measure at the distance between the suprailiac bone and the last rib with an accuracy of 0.5 cm at the beginning and end of the study. BMI will be calculated using the measured height and weight (weight (kg)/height (m²)).²⁸

Assessment of biochemical indicators

At the study baseline and after the intervention, 10 mL blood sample will be taken from each person in a fasting state (<12 hours) and serum will be separated from the whole blood by centrifugation for 10 min at 3500 rpm. Fasting plasma glucose levels and HbA1c will be measured at the sampling day using standard methods by the commercial kits (Pars Azmoun Co, Tehran, Iran). HbA1c will be measured in the whole blood by turbidimetry method using the AutoAnalyzer. For further analyses, the serum will be immediately frozen at -70°C until the relevant tests are performed. Serum TC and TG concentrations will be measured using laboratory methods through enzymes such as cholesterol esterase, cholesterol oxidase and glycerol phosphate oxidase, respectively, and using standard kits. HDL-cholesterol will be measured after the deposition of apolipoprotein B, which contains lipoproteins with phosphotungstic acid. LDL-cholesterol levels will be measured using Friedwald formulas.²⁹ In order to measure inflammatory factors, after separating the serum from the blood sample with the help of ELISA kit, the serums will be stored in a freezer at -80°C until the tests are performed. Serum levels of inflammatory factors including hs-CRP will be measured. Serum levels of inflammatory factor will be measured using a commercial ELISA kit based on the Sandwich ELISA method.

Renal function

Before and after the intervention, spot urine samples in the early morning will be collected from patients in a fasting state to examine biomarkers of renal function. Urinary

albumin will be quantified by Hitachi 902 AutoAnalyzer (Boehringer Mannheim, Germany). BUN and creatinine will be assessed using commercial kits available in the market (Pars Azmoun) based on a 10 mL blood samples that will be taken from each person in a 12-hour fasting state. The following formula will also be used to calculate eGFR and urine albumin-creatinine ratio (UACR).

$$eGFR = GFR = 141 \times \min(Scr/\kappa, 1)^\alpha \times \max(Scr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018 \text{ (if female)} \times 1.159 \text{ (if black)}.$$

Scr is serum creatinine (mg/dL), κ is 0.7 for female and 0.9 for male, α is -0.329 for female and -0.411 for male, min indicates the minimum of Scr/ κ or 1, and max indicates the maximum of Scr/ κ or 1.

UACR (U)=urine albumin (mg/dL)/urine creatinine (g/dL).

Blood pressure

Patients' blood pressure is measured from the right hand while sitting with the help of a mercury barometer calibrated by the Iranian Institute of Standards and Industrial Research. Patients will be asked to refrain from smoking, exercising or drinking tea/coffee for 1 hour before the measurement. They must also have an empty bladder before measuring their blood pressure. The patient's blood pressure is taken twice at a 5-minute interval while seated for 5 min. The pressure measured after hearing the first Korotkoff sound is equal to the systolic blood pressure, and the pressure measured after the Korotkoff sound fades is equal to the diastolic blood pressure. The average of the two measurements will be used to calculate the systolic and diastolic blood pressures.

Other variables

We will apply a general questionnaire to collect information about participants' age, sex, marital status (single/married), level of education (below diploma/higher or equal to diploma), supplement consumption status (yes/no), family history of the disease (diabetes; cardiovascular disease; cancer; liver disease; kidney, lung, thyroid disease; and central nervous system disorders), tobacco use (non-smoker/ex-smoker/current smoker), job (employee, worker, freelancer), duration of diabetes (years) and insulin injection (yes/no).

Adverse events

The adverse effects of the dietary intervention will be asked to be reported by study participants. This will be done in a weekly contact the investigators would have with study subjects. All reported adverse effects will be recorded and explained in the final report.

Statistical analysis

All analyses will be done using SPSS software V.26. First, we will apply the Kolmogorov-Smirnov test to examine the normality of the variable distribution. A logarithmic transformation will be used if the variable was non-normally distributed. The findings will be reported as means \pm SDs or percentages, as appropriate. Baseline characteristics of study participants will be reported separately

by two groups. Differences in food and nutrient intake throughout the intervention will be examined by the use of Student's t-test. The effects of probiotic yoghurt consumption on outcome variables will be assessed using repeated measures analysis of variance, in which we will take into account the differences in baseline levels of these variables as well as the probable difference in dietary intake throughout the intervention. In addition to considering means of variables in these analyses, changes in outcome variables following the intervention will also be assessed and reported. Furthermore, to simplify the effect of the intervention, we will also compute per cent changes in any outcome variable in the study. $P < 0.05$ will be considered as statistically significant.

Patient and public involvement

The trial design, rationale and planned intervention have been discussed and planned with consumers to ensure acceptability. Patients approached for recruitment will be asked to complete a short questionnaire to provide feedback on their concerns relating to nature of interventions they would accept. Those participating in the trial will also be asked for feedback on the trial intervention, the trial process and any difficulties encountered. Information regarding their attitudes towards the implementation of trial findings, if positive, will also be sought.

DISCUSSION

The average incidence of DN is high (3% annually) during the first 10–20 years following the onset of diabetes.³⁰ Hyperglycaemia, insulin resistance, oxidative stress and lipid disorders play an important role in the pathogenesis of DN.^{31–34} Dysbiosis (changes in the composition of intestinal microbiota) appears to be a sensitive factor in the developing and progressing chronic kidney disease.³⁵ Probiotics might play a key role in modulating intestinal bacterial flora and alteration of intestinal permeability.^{36,37} In addition, given the role of inflammation in the pathogenesis of DN, probiotics can ultimately lead to increased insulin sensitivity, glycaemic control and reduced inflammation through producing short-chain fatty acids.^{38,39} Despite a number of studies on the impact of probiotic supplements on renal function, only a few studies have investigated the impact of probiotic-enriched foods.^{1,40} However, some studies did not perform a blinded study and some others have used bacterial strains that are not routinely used in the production of probiotic-enriched foods. In the current study, we aimed to evaluate the effect of probiotic yoghurt consumption on albumin to creatinine ratio, eGFR and metabolic parameters in patients with type 2 diabetes with DN. In case of finding an appropriate effect of commercially available probiotic-enriched foods on outcome variables in the current study, the inclusion of these foods in the dietary management of patients with DN would be a cost-effective method to take advantage of probiotics.

Author affiliations

¹Department of Clinical Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran

²Endocrinology and Metabolism Research Center, Vali-Asr Hospital, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

³Department of Community Nutrition, Tehran University of Medical Sciences, Tehran, Iran

⁴Obesity and Eating Habits Research Center, Endocrinology and Metabolism Molecular -Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

Contributors AIE, KD, MN, AhE and NS contributed to design and developed methodology. SMG contributed to manuscript writing. AhE has reviewed the manuscript and validated the methodology. All authors read and approved the final manuscript.

Funding This study will be financially supported by a grant from Tehran University of Medical Sciences (TUMS), Tehran, Iran (award/grant number: not applicable).

Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iD

Seyed Mojtaba Ghoreishy <http://orcid.org/0000-0003-4215-6567>

REFERENCES

- Abbasi B, Ghiasvand R, Mirlohi M. Kidney function improvement by soy milk containing *Lactobacillus plantarum* A7 in type 2 diabetic patients with nephropathy: a double-blinded randomized controlled trial. *Iran J Kidney Dis* 2017;11:36–43.
- Bose M, Almas S, Prabhakar S. Wnt signaling and podocyte dysfunction in diabetic nephropathy. *J Investig Med* 2017;65:1093–101.
- Flyvbjerg A. The role of the complement system in diabetic nephropathy. *Nat Rev Nephrol* 2017;13:311–8.
- Qi C, Mao X, Zhang Z, *et al*. Classification and differential diagnosis of diabetic nephropathy. *J Diabetes Res* 2017;2017:1–7.
- Tazakori Z, Zare M, Jafarabadi MA. Probiotic yogurt effect on macronutrients ingredients, blood glucose and lipid profile in type 2 diabetes. *J Pak Med Assoc* 2017;67:1123.
- Papadopoulou-Marketou N, Kanaka-Gantenbein C, Marketos N, *et al*. Biomarkers of diabetic nephropathy: a 2017 update. *Crit Rev Clin Lab Sci* 2017;54:326–42.
- Satirapoj B. Tubulointerstitial biomarkers for diabetic nephropathy. *J Diabetes Res* 2018;2018:1–6.
- Sun H-J, Wu Z-Y, Cao L, *et al*. Hydrogen sulfide: recent progression and perspectives for the treatment of diabetic nephropathy. *Molecules* 2019;24:2857.
- Granados-Pineda J, Uribe-Urbe N, García-López P, *et al*. Effect of pinocembrin isolated from Mexican brown propolis on diabetic nephropathy. *Molecules* 2018;23:852.
- Oshima M, Toyama T, Haneda M, *et al*. Estimated glomerular filtration rate decline and risk of end-stage renal disease in type 2 diabetes. *PLoS One* 2018;13:e0201535.

- 11 Lee J, Kim D, Oh Y, *et al.* Lysophosphatidic acid signaling in diabetic nephropathy. *Int J Mol Sci* 2019;20:2850.
- 12 Fernandes R, Viana SD, Nunes S, *et al.* Diabetic gut microbiota dysbiosis as an inflammaging and immunosenescence condition that fosters progression of retinopathy and nephropathy. *Biochim Biophys Acta Mol Basis Dis* 2019;1865:1876–97.
- 13 Ramezani A, Massy ZA, Meijers B, *et al.* Role of the gut microbiome in uremia: a potential therapeutic target. *American Journal of Kidney Diseases* 2016;67:483–98.
- 14 Firouzi S, Mohd-Yusof B-N, Majid H-A, *et al.* Effect of microbial cell preparation on renal profile and liver function among type 2 diabetics: a randomized controlled trial. *BMC Complement Altern Med* 2015;15:433.
- 15 Mazruei Arani N, Emam-Djomeh Z, Tavakolipour H, *et al.* The effects of probiotic honey consumption on metabolic status in patients with diabetic nephropathy: a randomized, double-blind, controlled trial. *Probiotics Antimicrob Proteins* 2019;11:1195–201.
- 16 Miraghajani M, Zaghian N, dehkohneh A, *et al.* Probiotic soy milk consumption and renal function among type 2 diabetic patients with nephropathy: a randomized controlled clinical trial. *Probiotics Antimicrob Proteins* 2019;11:124–32.
- 17 Pei M, Wei L, Hu S, *et al.* Probiotics, prebiotics and synbiotics for chronic kidney disease: protocol for a systematic review and meta-analysis. *BMJ Open* 2018;8:e020863.
- 18 Soleimani A, Zarrati Mojarad M, Bahmani F, *et al.* Probiotic supplementation in diabetic hemodialysis patients has beneficial metabolic effects. *Kidney Int* 2017;91:435–42.
- 19 Borges NA, Carmo FL, Stockler-Pinto MB, *et al.* Probiotic supplementation in chronic kidney disease: a double-blind, randomized, placebo-controlled trial. *J Ren Nutr* 2018;28:28–36.
- 20 Graham K, Stack H, Rea R. Safety, beneficial and technological properties of enterococci for use in functional food applications – a review. *Crit Rev Food Sci Nutr* 2020;60:3836–61.
- 21 Wang X, Yang Y, Huycke MM. Risks associated with enterococci as probiotics. *Food Research International* 2020;129:108788.
- 22 Homayoni Rad A, Mehrabany EV, Alipoor B, *et al.* Do probiotics act more efficiently in foods than in supplements? *Nutrition* 2012;28:733.
- 23 Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, *et al.* Probiotic yogurt improves antioxidant status in type 2 diabetic patients. *Nutrition* 2012;28:539–43.
- 24 Haneda M, Utsunomiya K, Koya D, *et al.* A new classification of diabetic nephropathy 2014: a report from joint Committee on diabetic nephropathy. *Clin Exp Nephrol* 2015;19:1–5.
- 25 Farvid MS, Jalali M, Siassi F, *et al.* Comparison of the effects of vitamins and/or mineral supplementation on glomerular and tubular dysfunction in type 2 diabetes. *Diabetes Care* 2005;28:2458–64.
- 26 Noordzij M, Tripepi G, Dekker FW, *et al.* Sample size calculations: basic principles and common pitfalls. *Nephrology Dialysis Transplantation* 2010;25:1388–93.
- 27 Ainsworth BE, Haskell WL, Whitt MC. Compendium of physical activities: an update of activity codes and Met intensities. *Med Sci Sports Exerc* 2000;32:S498–516.
- 28 World Health Organization. *Obesity: preventing and managing the global epidemic*, 2000.
- 29 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499–502.
- 30 Magee C, Grieve DJ, Watson CJ, *et al.* Diabetic nephropathy: a tangled web to unweave. *Cardiovascular Drugs and Therapy* 2017;31:579–92.
- 31 Nakagawa T, Tanabe K, Croker BP, *et al.* Endothelial dysfunction as a potential contributor in diabetic nephropathy. *Nat Rev Nephrol* 2011;7:36–44.
- 32 Gnudi L, Coward RJM, Long DA. Diabetic nephropathy: perspective on novel molecular mechanisms. *Trends in Endocrinology & Metabolism* 2016;27:820–30.
- 33 Mendez MM, Folgado J, Tormo C, *et al.* Altered glutathione system is associated with the presence of distal symmetric peripheral polyneuropathy in type 2 diabetic subjects. *J Diabetes Complications* 2015;29:923–7.
- 34 Herder C, Lankisch M, Ziegler D, *et al.* Subclinical inflammation and diabetic polyneuropathy: MONICA/KORA survey F3 (Augsburg, Germany). *Diabetes Care* 2009;32:680–2.
- 35 Vaziri ND, Wong J, Pahl M, *et al.* Chronic kidney disease alters intestinal microbial flora. *Kidney Int* 2013;83:308–15.
- 36 Zhou M, Zhu J, Yu H, *et al.* Investigation into *in vitro* and *in vivo* models using intestinal epithelial IPEC-J2 cells and *Caenorhabditis elegans* for selecting probiotic candidates to control porcine enterotoxigenic *Escherichia coli*. *J Appl Microbiol* 2014;117:217–26.
- 37 Ranganathan N, Friedman EA, Tam P, *et al.* Probiotic dietary supplementation in patients with stage 3 and 4 chronic kidney disease: a 6-month pilot scale trial in Canada. *Curr Med Res Opin* 2009;25:1919–30.
- 38 Tilg H, Moschen AR, Food MAR. Food, immunity, and the microbiome. *Gastroenterology* 2015;148:1107–19.
- 39 Chen H, Yang X, Lu K, *et al.* Inhibition of high glucose-induced inflammation and fibrosis by a novel curcumin derivative prevents renal and heart injury in diabetic mice. *Toxicol Lett* 2017;278:48–58.
- 40 Miranda Alariste PV, Urbina Arronte R, Gómez Espinosa CO, *et al.* Effect of probiotics on human blood urea levels in patients with chronic renal failure. *Nutr Hosp* 2014;29:582–90.