ERA Operative Headquarters
Strada dei Mercati 16/A
43126 Parma, Italy
Tel: +39 0521 989078
fellowships@era-online.org
www.era-online.org

ERA Long-Term Research Fellowship Project

ERN & DESCARTES

Project's key info

Title of the project	**The Benzoic Acid and Nutritional and Clinical Health in Kidney Transplantation (BANCH) trial**
Working Group involved in the project	European Renal Nutrition Working Group (ERN) & Developing Education Science and Care for Renal Transplantation in European States Working Group (DESCARTES)
Principal Investigator(s) of the project	Stephan J.L. Bakker (The Netherlands) DESCARTES representative
Duration	12 months
Fellowship Grant	34.495,00 €
Start of the fellowship	Within 6 months after notification of the grant award to the fellow.

Receiving Institute

Name of receiving institute	University Medical Center Groningen
Supervisor's name	Stephan J.L. Bakker (The Netherlands) DESCARTES representative
Supervisor's e-mail address	s.j.l.bakker@umcg.nl DESCARTES representative

Project's detailed description

Project description
Background: Vitamin C is a small-molecular size, water-soluble molecule. Therefore, it would be expected that excess amounts of ingested vitamin C would be eliminated by urine and an inverse or absent association between kidney function and plasma vitamin C should be found. However, we have found in multiple cohorts of kidney transplant recipients (KTR) a positive association between eGFR and plasma vitamin C. We also found that plasma vitamin C concentration is higher among kidney donors that among transplant recipients. Furthermore, in an as yet unpublished follow-up study, we found that plasma vitamin C concentrations in healthy kidney donors become significantly lower rather than higher after donation of one kidney. These differences could not be explained by variation in vitamin C intake or intake of supplements containing vitamin C. We hypothesize that the cause of this rather counter-intuitive finding lies in the fact that with decreasing kidney function circulating concentrations of the also small-molecular size, water soluble substance benzoic acid (benzoate) will rise and will be better able to react with the circulating vitamin C. The result of these reactions being the production of the toxic metabolite benzene. Benzoic acid is a widely used preservative in food and beverages, commonly found in soft drinks, fruit juices, fermented vegetables, and high-sugar foods. Some products, such as lemonade and iced tea, contain 112-146 mg/L of benzoate, while emulsified sauces and

dressings can reach concentrations of 500-850 mg/kg. Due to its widespread use, concerns have been raised about the potential for benzoate to generate benzene when reacting with ascorbic acid. This reaction was first observed in vitro, particularly in beverages containing both preservatives, where traces of benzene were detected. As a result, food preservation guidelines have recommended e.g. replacing ascorbic acid with other antioxidants when using benzoate. While this reaction is commonly thought to occur only in vitro, animal studies suggest it may also occur in vivo. For instance, rat experiments demonstrated that benzoate exposure could lead to vitamin C deficiency. Benzene production from benzoate exposure is particularly concerning because benzene is associated with symptoms such as drowsiness, dizziness, headaches, and tremors, as well as serious health outcomes like severe anaemia and haematological malignancies. Additionally, benzene exposure could contribute to uremic symptoms and the increased malignancy risk observed in this population.
Understanding the risk of benzene exposure in patients with decreased kidney function is critical, particularly as benzoate use continues to rise, and vitamin C supplementation remains a topic of discussion for patients with chronic kidney disease and KTR.
This project aims to explore whether benzoate intake in KTR is associated with vitamin C deficiency and benzene exposure.

Research question: What is the association between benzoic acid intake, plasma vitamin C concentration and benzene exposition in kidney transplant recipients?

Hypothesis: Patients with impaired kidney function would have an impaired benzoate excretion leading to an increased in vivo reaction of benzoate with vitamin C and a consequent decreased plasma vitamin C concentration and an increased benzene exposition.

Methodology
Study design: Randomized crossover clinical trial
Population: Patients with a kidney transplantation who are currently in follow-up by the university medical centre Groningen (UMCG)
Inclusion criteria:

- Patients older than 18 years
- ≥1 year after kidney transplantation

Exclusion criteria:

- Patients who are unable to comprehend the instructions of the nutrition team, the questionnaires and tests
- Unable or unwilling to adhere to dietary recommendations of the study protocol
- Patients following a vegetarian diet
- Life expectancy ≤1 year

Exposure: 12 weeks of benzoic acid free diet.
There are multiple benzoate free diets described in literature (17), particularly for the treatment of orofacial granulomatosis. We will discuss these recommendations with the nutrition team of the UMCG and reach a final consensus regarding the recommendations that we will provided to patients during the intervention period. This diet will also be directed to (i) maintain total vitamin C intake stable, and to (ii) avoid changes in protein intake and green tea intake, which are two potential secondary sources of benzoate production in vivo (18).

Control: 12 weeks of regular diet.

Outcomes: To be evaluated in the 24-hours after the end of the 12 weeks intervention/control.
Primary:

- Plasma vitamin C concentration.

Secondary:

- Urinary Trans-muconic acid (ttMA) excretion.
- Adherence to the intervention, as evaluated by:
    - 24-hours dietary recall with subsequent benzoate intake calculation as described by Vandevijvere et al (6), and vitamin C intake calculation.
    - 24-hours urinary protein excretion with the subsequent calculation of total protein intake by the Maroni formula.
- Plasma creatinine concentration.
- Hemoglobin concentration.
- Total white blood cell count.
- Plasma malondialdehyde concentration.

Power calculation: In this cross-over study, we calculate that we will need 78 patients to detect a 7 mmolL difference in the plasma vitamin C concentration, given an standard deviation of 21.7 for the repeated measurements of the same individual and α of 0.05 and a β of 0.8

Role of the selected fellow: The fellow will be responsible for setting up and supervising the clinical trial. Furthermore, he or she will collect the outcome data, analyse the results and produce the respective scientific articles. The principal investigator (S.J.L.B) will handle the administrative setup to ensure ethical approval is obtained before the fellowship begins. This approach will enable the fellow to complete the project within the proposed year.

Goals of the project

Main goal: Determine whether in kidney transplant recipients benzoic acid intake leads to decreased plasma vitamin C concentration.

Secondary goals:
Assess whether in kidney transplant recipients benzoic acid intake leads to:

- Increased benzene exposition.
- Decreased haemoglobin.
- Decreased white cell count.
- Increased oxidative stress.

Expected end products: This research will lead to the production of at least 2 peer-reviewed publications.

Qualifications and/or expertise required to the fellow

Requirements include a Ph.D. (or equivalent) in clinical research, by preference in Nephrology. Mastery of English is also necessary. Desirable skills include experience with nutrition research in Nephrology and experience in the epidemiology field including study design, clinical data analysis and statistics.