

UNIVERSITÀ CATTOLICA DEL SACRO CUORE
Sede di Piacenza

Dottorato per il Sistema Agro-alimentare

Ph.D. in Agro-Food System

cycle XXXII

S.S.D: AGR/16

**Association between fatty acids profile and gut microbiota composition in
elderly patients with chronic kidney disease**

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Matriculation n.: 4612272

Academic Year 2018/2019



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Abstract

The aim of this thesis was to explore the possible associations between fatty acids (FA) profile and gut microbiota (gMb) with several conditions throughout the lifespan, from infancy to old age. In particular, we focused our attention on elderly subjects with Chronic Kidney Disease (CKD) and children with Acute Otitis Media (AOM).

The terms “Chronic Kidney Disease” refers to several disorders with a progressive kidney function decline. International guidelines approved the definition of CKD as a condition with the presence of markers of kidney damage or with the estimated glomerular filtration rate (eGFR) less than 60 ml/min/1.73 m² or both, for at least three months. End-stage renal disease is associated with a high cardiovascular disease (CVD) risk, the major cause of death in these patients. Chronic inflammation, oxidative stress, protein-energy wasting, disordered mineral metabolism, and deficiency of endogenous calcification inhibitors, known as non-traditional risks factor, take part in cardiovascular pathology in CKD. Inflammatory processes influence the physiological response to renal infection and injury but also participate in the development of potentially irreversible kidney damage with the production of various inflammatory molecular species, among whom eicosanoids and cytokines, from parental omega-6 long-chain polyunsaturated fatty acids (LCPUFA). Several studies focused their attention on the potential role of omega-3 (n-3) LCPUFA supplementation in subjects with CKD. Despite this, their effect on kidney damage is still not clear. However, it is widely agreed that a modified FA profile in CKD can determine a progression of the disease, inducing the inflammatory state. Moreover, high/normal n-3 LCPUFA levels decrease the risk of a decline of the disease. Omega-3 and omega-6 (n-6) LCPUFA concentrations and their ratios are tightly associated with renal health, because of their important roles in different pathways. Another aspect not very considered in the field of CKD is the role of circulating FA levels and their metabolites on the modulation of inflammation. The first aim of this study is to analyze the FA profile in elderly subjects with CKD. Blood samples have been collected from 57 subjects enrolled

in the study, and FA analysis has been performed. During the last years, several studies underlined the strong relationship between intestinal inflammation and adverse outcomes in CKD. The health of gastrointestinal tract is fundamental to ensure the well being of the host contributing to its nutrition, metabolism, physiology, and immune function. The bacterial communities colonizing humans have been seen in terms of mutualistic symbiosis with their hosts, a mutually beneficial coexistence, playing an important role in health and disease. Abnormal colonization or changes in the gut microbial composition determine dysbiosis, a state associated with different illnesses, such as obesity, type 2 diabetes, inflammatory bowel disease, cardiovascular disease, and also chronic kidney disease. The relationship between gut and kidney is a bi-directional relation with a mutual influence. Chronic kidney disease influences gMB characteristics, especially through high levels of urea that easily spread in the intestinal fluid where bacterial urease enzymes degrade it, then it is hydrolyzed in ammonium hydroxide that increases fecal pH with a consequent alteration of intestinal cellular junctions. Besides, high levels of urea change intestinal microbiota composition damaging permeability of intestinal barrier and promoting proteolysis with production and absorption of uremic toxins, such as indoxyl sulfate (IS) and p-cresol sulfate (p-CS). These toxins induce an inflammatory process associated with CKD. Under physiologic conditions, the kidney through the urine eliminates these compounds, but CKD patients have a compromised renal clearance. Therefore, these solutes tend to accumulate in the organs. IS and p-CS are tightly bound to human serum albumin (HSA), the most abundant plasma protein in the bloodstream. HSA is recognized as the main means of transport for endogenous and exogenous compounds, including fatty acids that seem to be the main endogenous ligand of HSA, multiple binding sites are used for monounsaturated fatty acids (MUFA) and PUFA. Thus, free fatty acids and uremic toxins compete for the same binding sites on HSA. It is important to assess fatty acid (FA) levels in patients with CKD because of the potential role to contrast the accumulation of uremic toxins derived from the intestinal bacterial community. As a consequence of this bi-directional relation between gut and kidney and the possible involvement of some compounds as metabolites of FA in the inflammatory

response, we investigate the correlation between circulating FA levels and the gMB composition in the same subjects with CKD, as the second aim of this thesis. 64 old CKD non-dialysis patients (eGFR 15-45 ml/min/1.73 m²) and 15 elderly subjects (>65 years) with normal renal function (eGFR >60 ml/min/1.73 m², CKD-EPI) are enrolled. Bacterial composition was studied in a previous observational study by denaturing gel gradient electrophoresis (DGGE), high-throughput sequencing (16S ribosomal RNA), and quantitative real-time PCR (qPCR). This study described an increased abundance of some bacteria associated with pathological conditions. In agreement with the literature, the author found a reduced abundance of saccharolytic and butyrate-producing bacteria (*Prevotella*, *Faecalibacterium prausnitzii*, *Roseburia*) in CKD patients respect to the control group. Butyrate plays a crucial role in the maintenance of the gut barrier function. Taking that into account, we decided to investigate the correlation between gMB composition and FA profile in these subjects. The main result of the study was the significant positive correlation between *Faecalibacterium prausnitzii* and total n-3 levels, both associated with the anti-inflammatory action. The present doctoral thesis underlines the need to perform further investigations in order to support evidence presented. Future studies may be useful to improve understanding of the effect of circulating fatty acids levels and their metabolites on gut microbial composition, inflammation process, and pathological conditions such as kidney disease. Our results showed that CKD patients with previous cardiovascular events had lower total and specific n-3 levels comparing with patients without them. Moreover, higher docosahexaenoic acid (DHA) levels and having had previous cardiovascular events seemed to have protective effects against further cardiovascular events. Moreover, we observed a significant reduction of the genera *Roseburia* and *Faecalibacterium* in CKD patients compared to C group and a significant lower abundance of *F. prausnitzii* and *Roseburia* spp. in CKD patients. Thus, our results seem in accordance with anti-inflammatory actions of total n-3, DHA, and saccharolytic and butyrate-producing bacteria. Many gMB changes seem to be related both to CKD and CVD. If the different gMB composition might play a causal role in cardiovascular events by an unbalanced production of

some toxic substances, or if the gMB changes are merely a consequence of different dietary and lifestyle behaviours of these patients, it cannot be explained by the present study and all the yet available data. Further studies, possibly utilizing new high-throughput tools, will be required to understand the potential correlations between the gMB composition and other inflammation and oxidative stress markers in these patients. Other two studies have been performed during the doctoral course, to reach a better comprehension of fatty acids, gut microbial community and inflammatory states. A prospective pilot clinical study has been performed to explore possible changes of gMB composition in children with AOM treated with amoxicillin with or without clavulanic acid. AOM is one of the most common bacterial infections in children and is normally treated with antibiotic therapies that lead to increasing antimicrobial resistance rates among otopathogens and may impair the correct development of the microbiota in early life. No significant differences were shown in the gMB composition of the overall cohort at different time intervals of the samples collection and in subjects treated with amoxicillin with or without clavulanic acid at different time intervals (T0, T1 and T2). A literature revision on lipids in infant formulae has been performed to better understanding quality and quality of dietary lipids because of their significant impact on health outcomes, especially when fat storing and/or absorption are limited (e.g., preterm birth and short bowel disease) or when fat byproducts may help to prevent some pathologies. The lipid composition of infant formulae varies according to the different fat sources used, and the potential biological effects are related to the variety of saturated and unsaturated FAs. Instead, ruminant-derived *trans* FAs and metabolites of n-3 LCPUFA with their anti-inflammatory properties can modulate immune function. Furthermore, dietary fats may influence the nutrient profile of formulae, improving the acceptance of these products and the compliance with dietary schedules. During the doctoral course, I spent a period abroad at Dell Pediatric Research Institute (DPRI), The University of Texas at Austin. In particular, I attended the laboratory of Doctor Brenna. I focused my research activity on a specific regulatory insertion-deletion polymorphism in the FADS gene cluster for better understanding its influence on PUFA and lipid profile.