



# The Role of Gut Dysbiosis in Malnutrition Mechanism in CKD-5 HD Patients

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## Abstract

Edited by: <https://publons.com/researcher/391987/mirko-spiroski/>

Citation: Widiastih E, Subagio HW, Lestariningsih L. The Role of Gut Dysbiosis in Malnutrition Mechanism in CKD-5 HD Patients. Open Access Maced J Med Sci. 2022 May 20; 10(E):1016-1023. <https://doi.org/10.3889/oamjms.2022.9870>

Keywords: Gut dysbiosis; Malnutrition mechanism; CKD-5 HD patients

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Received: 18-Apr-2022

Revised: 09-May-2022

Accepted: 10-May-2022

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

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Patients with terminal stage chronic kidney disease who have undergone hemodialysis (PGK-5 HD) have a high risk of developing malnutrition, which is characterized by wasting protein-energy and micronutrient deficiencies. Studies show a high prevalence of malnutrition in CKD-5 HD patients. The pathogenic mechanisms of malnutrition in CKD-5 HD are complex and involve the interaction of several pathophysiological changes including decreased appetite and nutrient intake, hormonal disturbances, metabolic imbalances, inflammation, increased catabolism, and abnormalities associated with dialysis action. A clear understanding of the pathophysiological mechanisms involved in the development of malnutrition in CKD-5 HD is required to develop strategies and interventions that are appropriate, effective, and reduce negative clinical outcomes. This article is a review of the pathophysiological mechanisms of malnutrition in CKD-5 HD patients caused by chronic inflammation due to intestinal dysbiosis.

## Introduction

The prevalence of CKD-5 HD patients was more than 2.5 million people worldwide in 2010 and is expected to increase to 5.4 million in 2030 with the largest proportion in Asians [1], [2]. The number of CKD-5 HD patients in Indonesia has increased significantly from year to year. Data from the Indonesia Renal Registry (IRR) in 2018 recorded that the number of new PGK-5 HD patients was 66,433 and the number of active HD patients was 132,142, an increase of more than 2 times compared to 2017 [3]. Morbidity and mortality of CKD-5 HD patients are reported to remain high despite the rapid development of HD management [3]. Chronic inflammation is known to have an important role in the pathophysiology of complications that support the high rates of morbidity and mortality in CKD-5 HD [4]. Recent studies have found and proved that digestive tract problems are a source of chronic inflammation in CKD-5 HD [5].

## Human Intestinal Microbiota Physiology

The human gastrointestinal system has about 100 trillion microbiota consisting of more than 1000 different species. The gut microbiota is a complex assemblage consisting of bacteria, archaea, viruses, and fungi. Intestinal microbiota play a role in regulating biological and physiological processes of the body in a state of eubiosis, which is a balanced status between bacterial populations in the gastrointestinal tract. The gut microbiota are able to carry out various important functions in maintaining overall body health. Intestinal dysbiosis is defined as a condition of imbalance between the microbiota population in the gastrointestinal tract, causing various health problems [6], [7].

The development of the human gut microbiota begins when humans are born. The gut microbiota of babies born by vaginal delivery are similar to the microbiota species in the mother's vagina in the early 20 minutes of life. The dominant species of gut microbiota found in infancy are *Lactobacillus* sp. and

*Prevotella* sp. There are differences between species of gut microbiota born by normal delivery and caesarean section. Intestinal microbiota in infants born by *cesarean section*, namely, *Clostridium* sp., *Staphylococcus* sp., *Propionibacterium* sp., and *Corynebacterium* sp. [6], [7]. As we age, there will be changes in the gut microbiota the baby because it is influenced by breast milk (ASI), the introduction of complementary foods, fever or infection, and the use of drugs [6], [7].

Healthy individuals have a composition of gut microbiota that consists of five phyla; *Firmicutes* and *Bacteroidetes* (collectively 90%), followed by *Actinobacteria*, *Verrucomicrobia*, and *Proteobacteria* in the lowest proportions. The gut microbiota play a fundamental role in the process of breaking down indigestible plant polysaccharides from food, biosynthesis of short-chain free fatty acids (SCFAs), biosynthesis of vitamins, biotransformation of conjugated bile acids, degradation of dietary oxalate, defense of the immune system, and reducing allergic responses to food and/or environmental antigens [6], [7].

Research shows that the gut microbiota increase the absorption and storage of lipids, thereby facilitating body's absorption necessary vitamins such as Vitamin K. Dysfunction of the gut microbiota can lead to diseases such as autoimmune diseases (diabetes, rheumatoid arthritis, muscular dystrophy, *multiple sclerosis*, and fibromyalgia). The accumulation of pathogenic microbes will cause changes in gene and metabolic activity [8].

## Changes in Intestinal Microbiota in Patients with CKD-5 HD

### *Intestinal microbiota*

A healthy and balanced diet is basically saccharolytic, the amount of saccharolytic and proteolytic is mainly regulated by the ratio of carbohydrates to nitrogen. Digestive disorders and protein absorption in CKD patients cause the amount of protein that reaches the gastrointestinal tract to be greater, and the effect of limiting fiber-rich foods to avoid hyperkalemia makes bacterial metabolism in CKD shift to the dominance of proteolytic bacteria [10], [11], [12]. The accumulation of urea in the circulation causes massive reflux into the digestive tract. Urea is hydrolyzed to ammonia by bacterial urease in the gastrointestinal lumen. The ammonia is then converted to ammonium hydroxide, which increases the gastrointestinal luminal pH. In addition to urea, large amounts of uric acid and oxalate are also secreted into the gastrointestinal lumen as an adaptive response to decreased renal excretion in CKD-5 HD patients [13], [14]. Various drugs used in PGK-5 HD can also affect the gastrointestinal microbiota [13], [14].

The total number of bacteria in feces has previously been reported to be lower in CKD patients compared to healthy controls. Vaziri *et al.* examined bacterial species in feces of PGK-5 HD patients and control subjects according to age, sex, and ethnicity using *phylogenetic microarray techniques*. Significant differences were found in the phylum *Actinobacteria*, *Firmicutes*, and *Proteobacteria* (urease-producing bacteria) in CKD-5 HD patients [15]. Wong *et al.* investigated the effects of comorbidity, diet, and medication on gut microbiota composition in CKD rats undergoing 5/6 nephrectomy, and important findings were obtained in the form of a decrease in the composition of the *Lactobacillaceae* and *Prevotellaceae* families. Secondary analysis of a study using the literature identified a significant expansion of bacterial urease (such as *Proteobacteria*, *Actinomycetales*, and *Clostridiaceae*) and *uricase* (*Proteobacteria* and *Actinomycetales*) in CKD-5 HD patients, it was also found that bacteria possessing indole-forming enzymes and p-Cresols (*Clostridiaceae* and *Enterobacteriaceae*) were increased, while those with butyrate-forming enzymes (*Lactobacillaceae* and *Prevotellaceae*) were reduced [16]. SCFA in feces, especially butyrate, was significantly lower in CKD-5 HD patients than in healthy controls [16].

## Pathophysiology of Intestinal Dysbiosis Causes Chronic Inflammation in CKD-5 HD Patients

The gastrointestinal tract has been suggested as a major trigger of systemic inflammation in CKD-5 HD patients in recent years [17]. *Postmortem* studies found inflammation throughout the gastrointestinal lumen wall in chronic dialysis patients [17]. Gastrointestinal inflammation further enhances the degradation of the epithelial barrier through the induction of tight junction protein endocytosis. Decreased tissue levels of the anti-inflammatory transcription factor Nrf2 (nuclear erythroid factor 2-related factor-2) further contribute to tight junction damage, eventually leading to translocation of bacteria, endotoxins, and uremic toxins from the gastrointestinal lumen into the circulation promoting systemic inflammation and non-traditional risks that are known to cause death from cardiovascular disorders and promote the progression of CKD [17].

Increased concentrations of uremic toxin in the gut of CKD-5 HD patients cause intestinal dysbiosis due to overgrowth of pathogenic bacteria. Overgrowth of pathogenic bacteria leads to loss of the integrity of the epithelial barrier [18]. Translocation of bacteria and bacterial components triggers the gastrointestinal immune system induces a pro-inflammatory response secreting IL-1 and IL-6 from intestinal epithelial cells,

promoting TH1 and TH17 responses by dendritic cells and macrophages generate higher commensal-specific IgG by B cells. In this context, binding of LPS to its receptor complex on macrophages results in increased production of inflammatory cytokines including IFN-, *IFN-*, *IL -1*, *IL-6*, *TNF*, and *IL-12*. Subclinical endotoxemia is a potential cause of inflammation in PGK-5 HD. Irregular immune response and chronic pro-inflammatory cytokine production lead to systemic inflammation, which can further worsen the clinical condition of CKD-5 HD patients and trigger the development of cardiovascular disease complications [18].

Progressive CKD will cause blood urea levels to increase so that more urea enters the gastrointestinal lumen, and so on, a vicious circle in the *gut-kidney axis* which further aggravates the chronic inflammatory process in CKD patients [17].

Chronic inflammation was first recognized 20 years ago as a major component of the uremic phenotype associated with cardiovascular disease and *protein-energy wasting* (PEW) and is used as a strong predictor of mortality in CKD-5 HD patients [19]. The pathophysiology involved in the development of chronic inflammation in PGK-5 HD is multifactorial, including [17], [19]:

1. Exogenous factors such as dialysis membranes and central venous catheters;
2. Cellular factors, such as oxidative stress and cellular *aging*;
3. Tissue factors, such as hypoxia, fluid overload, and sodium overload;
4. *Microbiota* factors, such as immune dysfunction and *gut dysbiosis*; and
5. Retention of uremic toxins, such as indoxyl sulfate, AGES products, and *calcioprotein particles*.

The inflammatory process is a protective physiological mechanism in host defense against infection, tissue repair response, adaptation to stress, and restoration of homeostasis. The controlled inflammatory response benefits the host by eradicating noxious stimuli and initiating the healing process in the tissues; but it can also be detrimental if deregulated. Persistent inflammation is thought to contribute to ongoing complications including arteriosclerosis, atherosclerosis, osteoporosis, weakness, malnutrition (PEW), diabetes, depression, and constipation, which are complications of chronic inflammation that accompany CKD-5 HD [17], [19].

## Malnutrition in CKD-5 HD Patients

A clear understanding of the pathophysiological mechanisms involved in the mechanism of malnutrition

in CKD-5 HD is needed to develop effective strategies and interventions that will reduce negative clinical outcomes [20]. Based on clinical parameters, a malnutrition prevalence rate of 20–45% has been reported in various studies in children with CKD. A study using the global subjective rating scale (SGA) found a prevalence of malnutrition of 31% of adults with CKD, including dialysis and non-dialysis patients [20], [21].

Malnutrition in CKD is not entirely caused by a decrease in nutritional intake. The combination of several risk factors for malnutrition, including inflammation, hormonal imbalance, decreased appetite and food intake, increased catabolism, loss of nutrients in the dialysate, and metabolic derangements causes chronic kidney disease patients to be malnourished.

The American Society for Parenteral and Enteral Nutrition (ASPEN) defines malnutrition as an imbalance between nutritional requirements and intake that results in a cumulative deficit of energy, protein, or micronutrients that can negatively impact growth, development, and other relevant outcomes. This definition assumes a state of malnutrition, which is a waste of protein-energy and a deficiency of micronutrients.

The International Society of Renal Nutrition and Metabolism (ISRNM) recommends the term malnutrition in CKD as protein-energy wasting (PEW). PEW is defined as a state of decreased protein stores and energy fuel (i.e., body protein and fat mass). This term was proposed by ISRNM in 2008 to specifically refer to a state of decreased protein and body fat stores (*wasting*). The difference with protein-energy malnutrition is a form of pure protein-energy wastage characterized by insufficient food intake. PEW cannot be improved by simply increasing/improving energy intake

## Pathophysiology of PEW in PGK-5 HD

The pathogenesis of PEW is multifactorial. Decreased protein intake and energy intake due to anorexia, increased protein catabolism, decreased anabolism, chronic inflammation, metabolic acidosis, and hormonal imbalance are all. Anorexia often occurs in CKD patients and can be caused by changes in *orexigenic* (appetite stimulating) and *anorexigenic* (appetite inhibiting) hormones, accumulation of metabolic waste products in the body in kidney failure, abnormal taste, and the effect of drugs on the sense of taste. The cumulative impact of these factors results in a decrease in nutritional intake. The result of the chronic inflammatory state in CKD is an increase in residual energy expenditure (REE), which increases protein catabolism and decreases anabolism. Studies have shown an increase in resting energy expenditure

ranging from 12 to 20% during dialysis, indicating increased protein requirements and energy intake in dialysis patients. Protein catabolism, in addition to increased loss of protein (mostly amino acids) through dialysis techniques (both hemodialysis and peritoneal dialysis) and decreased albumin synthesis, leads to negative nitrogen balance and muscle disassembly in the body [20], [22].

The body's metabolism in CKD is significantly altered due to the progressive accumulation of metabolic by-products that the kidneys naturally have to clear. Metabolic disorders such as metabolic acidosis, hyperparathyroidism, insulin resistance, upregulation of the renin-angiotensin-aldosterone system, and dyslipidemia are common in CKD. Metabolic acidosis occurs early in CKD because of reduced excretion of the acid load produced by metabolic activity. Various studies have shown an association between metabolic acidosis and increased protein catabolism in patients with CKD [20], [22], [23].

Proteolysis induced by regulation of the ubiquitin-proteasome system, also facilitates whole-body protein degradation. Metabolic acidosis, chronic inflammation, insulin resistance, and elevated angiotensin II levels, all of which are seen in CKD, also stimulate the ubiquitin-proteasome enzyme system. As a result, muscle wasting and malnutrition ensue [22], [24]. Uremic toxin, which is progressively retained in CKD, is also known to inhibit lipoprotein lipase and hepatic lipase resulting in less lipid degradation and dyslipidemia [20], [25]. Correction of metabolic disorders through provision of adequate dialysis and use of available medical therapies is an important treatment strategy in the management of malnutrition in CKD [26].

Patients with terminal stage chronic kidney disease who have undergone hemodialysis (PGK-5 HD) have a high risk of developing malnutrition, which is characterized by wasting protein-energy and micronutrient deficiencies. Studies show a high prevalence of malnutrition in CKD-5 HD patients. The pathogenic mechanisms of malnutrition in CKD-5 HD are complex and involve the interaction of several pathophysiological changes. A clear understanding of the pathophysiological mechanisms involved in the development of malnutrition in CKD-5 HD is required to develop strategies and interventions that are appropriate, effective, and reduce negative clinical outcomes. This article is a review of the pathophysiological mechanisms of malnutrition in CKD-5 HD patients caused by chronic inflammation due to intestinal dysbiosis.

Chronic systemic inflammation is very common in patients with CKD and is associated with an increased disease burden. Inflammatory markers including the pro-inflammatory cytokines IL-6, IL-1 $\beta$ , IL-18, IL-6, TNF $\alpha$ , IL-8, and CRP are elevated in patients with CKD and have been associated with increased mortality in these patients. Hypoalbuminemia and elevated

ferritin levels are other markers of inflammation, with hypoalbuminemia being a strong predictor of death in these patients [22]. Chronic inflammatory states induce anorexia, reduce protein and calorie intake, and reduce albumin synthesis leading to protein-energy wasting and hypoalbuminemia. A comprehensive and multifaceted approach to the treatment of inflammation in CKD patients can reduce malnutrition and provide better outcomes [22], [24].

Delayed gastric emptying (gastroparesis) is common in CKD patients, as shown in several studies looking at gastric emptying. Elevated levels of gastrointestinal hormones including gastrin, cholecystokinin, and gastric inhibitory polypeptides can alter gastric motor function in CKD patients [13], [20]. Gastroparesis is associated with gastrointestinal symptoms such as dyspepsia, early satiety, bloating, vomiting, and gastroesophageal reflux. In addition to the stomach, this condition is also involved in slowing gastrointestinal peristalsis, thereby increasing complaints of constipation in CKD patients. These symptoms contribute to a significant reduction in nutrient intake and are an important cause of malnutrition in patients with chronic kidney disease [13], [20].

Dialysis techniques have been known to contribute to nutritional deficits in several ways. Hemodialysis patients had higher CRP levels, inflammation, oxidative stress, and increased muscle protein breakdown when compared to other CKD patients. This is attributed to the induction of flow in the inflammatory pathway when blood comes into contact with the dialyzers membrane. High inflammation can trigger anorexia and proteolysis as well as tissue lipolysis. As previously discussed, albumin loss is increased in peritoneal dialysis, placing these patients at greater risk for protein-energy malnutrition. On the other hand, suboptimal dialysis can lead to decreased clearance of uremic toxins, leading to malnutrition in the patient [13], [20].

Various studies have investigated the impact of dialysis dose on the nutritional status of dialysis patients and found improvements in the nutritional status of patients receiving more dialysis. At a minimum, patients receiving dialysis should be given the KDOQI recommended dialysis dose for hemodialysis and peritoneal dialysis to avoid *underdialysis* [20], [26], [27].

## Role of Chronic Inflammation in PEW

Management of the prevention of complications in patients with CKD-5 HD continues to progress, but muscle wasting is still an unresolved problem. Muscle wasting is defined as unintentional weight loss, which can be divided into loss of lean body mass and fat mass,



and has been recognized as a common and major problem of CKD affecting daily activities, quality of life, immune function, number of hospitalization days, and even death [28]. In the general population, high body mass has been shown to be a conventional risk factor for cardiovascular events and all-cause mortality [28].

Muscle wasting in CKD-5 HD patients occurs through accelerated protein degradation or decreased protein synthesis. Protein homeostasis between synthesis and degradation depends on protein intake and utilization. Approximately 4 g protein/kg body weight is synthesized and degraded per day in normal adults [28]. Skeletal muscle is a dynamic organ and is the largest reservoir of protein, consisting of amino acids and carbon chains. Muscle mass is the most reliable indicator of protein homeostasis and is affected by various clinical catabolic diseases such as stress, liver failure, cancer, sepsis, diabetes, and CKD [28]. Accelerated protein degradation without an adequate protein supply can lead to skeletal muscle atrophy, leading to muscle wasting [28].

The mechanisms of *muscle wasting* are complex and several of them have been documented to explain muscle wasting in CKD patients with and without HD [28]. TNF- $\alpha$  (TNF-Alfa) has long been associated with muscle pathology and was originally called “*cachectin*” for its catabolic effects. Experimental animals lose muscle mass when intervened with TNF- or when exposed to interventions that increase endogenous TNF- (e.g., sepsis or tumor implantation). In humans, muscle catabolism has been associated with TNF- in inflammatory diseases including cancer, congestive heart failure, AIDS, and chronic obstructive pulmonary disease (COPD). Malnourished individuals with COPD have elevated serum levels of TNF- which reflects TNF- production excessive by peripheral blood monocytes. Loss of muscle mass contributes to weakness, fatigue, and loss of mobility for individuals with critical illness and other inflammatory diseases [28].

The cellular mechanism of the action of TNF- causing muscle wasting is shown in Figure 1 [28]. TNF- binding to the TNF- receptor type 1 (TNFR1) stimulates increased production of reactive oxygen species (ROS) by mitochondrial electron transport, thereby activating nuclear factor- $\kappa$ B (NF- $\kappa$ B). Furthermore, NF- $\kappa$ B enhances the activity of the ubiquitin (Ubq)/proteasome pathway, accelerating protein degradation. TNF- $\alpha$  alters circulating levels of hormones that regulate muscle synthesis and affect tissue sensitivity to these factors. TNF- also stimulates the production of catabolic cytokines and induces anorexia [28].

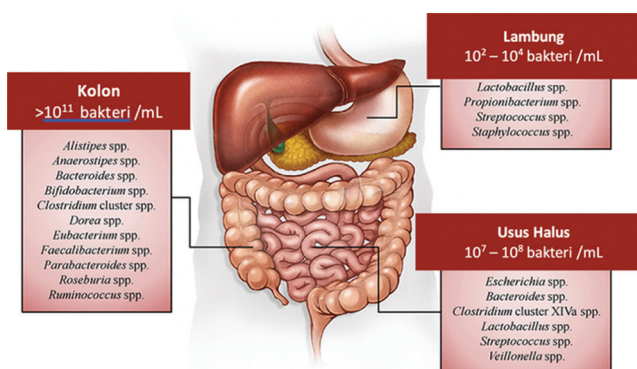


Figure 1: Bacterial composition in stomach, small intestine and colon based on 16S r-RNA gene sequence studies. Modified from Rivière et al. *Bifidobacteria and butyrate-producing colon bacteria: importance and strategies for their stimulation in the human gut*. *Front Microbiol.* 2016;7:11-9 [9].

TNF- can cause weight loss through two different mechanisms, namely, by influencing central body weight regulation in the brain and by causing catabolic processes in the body's periphery. Therefore, inhibition of TNF- can affect both central and peripheral mechanisms that regulate body weight [29]. TNF- and other pro-inflammatory cytokines such as interleukin (IL)-1 $\beta$  and IL-6 have been shown to induce loss of appetite by hypothalamic anorexigenic signaling. Mechanistic studies suggest that this appetite and weight loss are the result of cytokines that stimulate the production and release of anorexigenic neuropeptides, such as corticotropin-releasing factor, and inhibit signaling with the orexigenic neuropeptide Y (NPY) network [29]. These results are in line with experimental, preclinical, and clinical studies showing that IL-1 $\beta$ , IL-6, and TNF- induce appetite and weight loss when administered peripherally or directly to the brain. Metsios *et al.* investigated changes in physical activity and protein intake in patients receiving TNF- inhibitors and found that both were increased. Therefore, the possibility of weight gain and BMI is the result of increased calorie consumption due to the return of normal appetite [29].

Inflammation is also a major consequence of CKD and HD, and many inflammatory mediators have been shown to modulate insulin-associated signaling pathways in skeletal muscle. Inflammatory factors such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) suppresses insulin receptor signaling through inhibition and degradation of IRS in skeletal muscle. In addition, TNF- activates caspase-3 and NF- $\kappa$ B, which stimulates UPS activation, leading to muscle wasting [28].

## How to Assess PEW in CK-5 HD Patients

Routine PEW screening in CKD-5 HD patients is rarely performed because accurate determination of nutritional status requires procedures such as anthropometry, body composition, and biochemical

measurements; and functional, dietary, and subjective assessments are time consuming, cost effective, and inconvenient for most dialysis centers. Subjective global assessment (SGA) is the most commonly used method but is only semi-quantitative and has limited reliability and precision. Subsequent developments created a fully quantitative nutritional assessment system, using a modified SGA, DMS, and MIS which were developed by combining the advantages of SGA and extending its reliability and precision [23], [30].

MIS consists of four parts, namely, nutritional history, physical examination, BMI, and laboratory parameters. This score indicates four levels of severity ranging from 0 (normal) to 3 (very abnormal). Values of the 10 components each ranged from 0 (normal) to 30 (heavy PEW). A higher score reflects the level of malnutrition and severe inflammation. In the study of Kalantar-Zadeh *et al.*, this score was closely related to inflammation, body fat percentage and frequency of hospitalization, and mortality [23], [30].

DMS consists of seven variables such as changes in body weight, food intake, gastrointestinal symptoms, functional capacity, comorbidities, decreased fat stores, and signs of muscle wasting. MIS, on the other hand, includes seven components of DMS plus three new components, namely, BMI, serum albumin, and TIBC [30]. Both DMS and MIS are significantly correlated in hemodialysis patients and are valid tools to use for nutritional screening, also having the advantage of detecting small changes in nutritional status over time which can guide clinicians for the assessment of nutritional interventions. DMS and MIS had a sensitivity of 94% and 87% and a specificity of 88% and 96%, respectively, compared to SGA [30]. With this tool, malnutrition can be easily detected in minutes. DMS is a more practical and simpler tool for detecting malnutrition in routine hospital assessments [30].

## How to Analyze Intestinal Dysbiosis in PGK-5 HD Patients

The number of microbial cells in the human body is much greater than the number of human cells [7]. These bacterial communities play important roles, such as aiding in the digestion of food, synthesizing necessary vitamins, and assisting the immune system in defending our bodies against invading pathogens [31], [32]. The advent of the era of *omics*-based systems biology has opened up new scenarios in the understanding of the gut ecosystem by elucidating its shape, modulation, and interaction with microorganisms, the function of food, and the role of nutrients in health. *Omic*s technology is currently being applied to determine disease-specific markers and novel diagnostic targets, find functional changes in the physiopathology of several diseases,

discover the relationship of gut microbiota, and host metabolism [33], [34].

There are three omic approaches that have been developed, namely, metagenomics, metatranscriptomics, and metabolomics to obtain useful information for understanding the microbiome [33]. Metagenomics is the study of the genome in a microbial community and is the first step to studying the microbiome [33]. Metagenomics *its main* aim is to infer the taxonomic profile of the microbial community. Although whole-metagenome sequencing (WMS) provides a glimpse of the functional profile of the microbial community, it would be better to infer it using metatranscriptomics involving complete transcriptome (meta) sequencing of the microbial community [33]. Metatranscriptomics tells us about the genes expressed by the community as a whole. Metatranscriptomics using functional annotations of expressed genes, it is possible to infer the functional profile of a community under certain conditions, which usually depend on the status of the host [33]. Metagenomics helps answer the question “*what is the composition of microbial communities under different conditions?*” hence, metatranscriptomics helps answer the question “*what genes are collectively expressed under different conditions?*” the question metabolomics considers is “*what byproducts are produced? under different conditions?*” [33]

One of the popular marker genes used in metagenomic studies is 16S ribosomal RNA (rRNA) sequencing analysis [33]. Metabolomics is a comprehensive analysis in which all subject metabolites (small molecules released by organisms into the immediate environment) are identified and measured. Metabolome is considered to be the most direct indicator of changes in homeostasis (i.e., *intestinal dysbiosis*). The application of metabolomics to drug discovery and pharmacogenomics is a promising avenue for treatment. Metabolic profiles associated with the microbiome may exhibit strong dependence on environmental factors (e.g., diet, exposure to xenobiotics, and environmental stressors), providing valuable information not only about the characteristics of the microbiome but also about the interactions of microbial communities with the host environment [33].

Metabolomics aims to improve our understanding of the role of the microbiome in the transformation of nutrients and pollutants as well as other abiotic factors that may affect the homeostasis of the host environment. Microbial communities exert a strong influence on critical biogeochemical cycles, and studies of their metabolism can help develop predictive biomarkers for environmental stressors [33]. Generating metabolomic data differ significantly from generating metagenomic and metatranscriptomic data, which are highly sequencing dependent. The identification and quantification of metabolites is usually carried out using a combination of chromatographic techniques, namely, gas chromatography (GC), liquid chromatography (LC),

and detection methods, such as mass spectrometry (MS) and nuclear magnetic resonance (NMR) [33]. The results of a review by Primec *et al.* (2017) found that for the fecal analysis of SCFA, the best for now is to use gas chromatography (GC) [35], [36].

The metabolomics approach has been applied to several studies on *the gut microbiota*, mostly focusing on the exploration of disease-associated metabolites to obtain detailed information on intestinal metabolic pathways. The study of its composition helps to distinguish between unhealthy and healthy subjects. In addition, the identification of metabolites can highlight how lifestyle and dietary habits influence certain disease conditions [33], [35]. *Fecal SCFA* concentration is a metabolomic analysis that can be used to analyze the composition of the *gut microbiota* based on the bacterial metabolites it produces [37]. A study by Chai *et al.* found that the number of *fecal SCFA* decreased according to changes in the *gut microbiota profile as a result of 16s-RNA sequencing* tests in patients with nephropathy [38]. A study by Wang *et al.* in CKD patients found that *fecal SCFA* levels were reduced in CKD patients [39].

## Conclusion

The high rate of morbidity and mortality in CKD-5 HD patients is caused by a chronic inflammatory process that is multifactorial and recent studies have found that the imbalance of gut microbiota is one of the main sources of chronic inflammation. Chronic inflammatory states lead to cardiovascular disorders. Chronic inflammation induces anorexia, reduces protein and calorie intake, and reduces albumin synthesis, leading to protein-energy wasting and hypoalbuminemia. A comprehensive and multifaceted approach to the treatment of inflammation in CKD-5 HD patients can reduce cardiovascular complications and prevent malnutrition in CKD-5 HD patients. Interventions that aim to properly restore gastrointestinal eubiosis are able to slow the progression of CKD and reduce chronic inflammatory processes in CKD-5 HD patients.

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