Quantitative Reduction of Gut Microbiota-Derived Short-Chain Fatty Acids in Stool and Serum in Diabetic Kidney Disease

Chenyu Zhong
Department of Nephropathy, Ningbo Huamei Hospital University of Chinese Academy of Sciences;Ningbo Institute of Life and Health Industry,University of Chinese Academy of Sciences,Ningbo,China

Zhiwei Dai
Department of Nephropathy,HwaMei Hospital, University of Chinese Academy of Sciences;Ningbo Institute of Life and Health Industry,University of Chinese Academy of Sciences,Ningbo,China

Lingxiong Chai
Department of Nephropathy,HwaMei Hospital, University of Chinese Academy of Sciences;Ningbo Institute of Life and Health Industry,University of Chinese Academy of Sciences, Ningbo,China

Lingping Wu
Department of Nephropathy, HwaMei Hospital, University of Chinese Academy of Sciences; Ningbo Institute of Life and Health Industry,University of Chinese Academy of Science,Ningbo,China

Jianhui Li
Department of Endocrinology, HwaMei Hospital, University of Chinese Academy of Sciences; Ningbo Institute of Life and Health Industry, University of Chinese Academy of Sciences,Ningbo, China

Weiying Guo
Department of Endocrinology, HwaMei Hospital, University of Chinese Academy of Sciences;Ningbo Institute of Life and Health Industry,University of Chinese Academy of Sciences,Ningbo, China

Jie Zhang
Department of Endocrinology, HwaMei Hospital,University of Chinese Academy of Science;Ningbo Institute of Life and Health Industry,University of Chinese Academy of Sciences,Ningbo, China

Qun Zhang
Department of Endocrinology,HwaMei Hospital, University of Chinese Academy of Sciences;Ningbo Institute of Life and Health Industry,University of Chinese Academy of Sciences,Ningbo,China

Congping Xue
Department of Nephropathy,HwaMei Hospital,University of Chinese Academy of Sciences;Ningbo Institute of Life and Health Industry,University of Chinese Academy of Sciences,Ningbo,China

Haixue Lin
Department of Nephropathy,HwaMei Hospital,University of Chinese Academy of Sciences;Ningbo Institute of Life and Health Industry,University of Chinese Academy of Sciences,Ningbo,China

Qun Luo
Department of Nephropathy,HwaMei Hospital,University of Chinese Academy of Sciences;Ningbo Institute of Life and Health Industry,University of Chinese Academy of Sciences,Ningbo,China

kedan cai (caikedan@ucas.edu.cn)
HwaMei Hospital, University of Chinese Academy of Sciences https://orcid.org/0000-0003-3169-5143

Research

Keywords: diabetes mellitus, diabetic kidney disease, gastrointestinal microbiome, short-chain fatty acids

Posted Date: April 6th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-378090/v1

Abstract

Objectives

Previous studies found the dysbiosis of intestinal microbiota in individuals with diabetic kidney disease (DKD),especially the decreased SCFA-producing bacteria. We aimed to investigate stool and serum short-chain fatty acids (SCFAs), gut microbiota-derived metabolites, in individuals with DKD and the correlations.

Methods

A total of 30 participants with DKD, 30 participants with type 2 diabetes mellitus (DM) and 30 normal controls (NC) in HwaMei Hospital were recruited from 1/1/2018 to 12/31/2019. Participants with DKD were divided into low estimated glomerular filtration rate (eGFR) (eGFR<60ml/min, n=14) and high eGFR (eGFR≥60ml/min, n=16) subgroups. Stool and serum were measured for SCFAs with gas chromatograph-mass spectrometry.

Results

The group with DKD showed markedly lower levels of fecal acetate, propionate and butyrate versus NC group (P<0.05), and the lowest fecal total SCFAs concentration among the the groups. The group with DKD also had a lower serum caproate concentration than that with diabetes (P<0.05). In the univariate regression analysis, fecal and serum acetate correlated with eGFR in the group with DKD (OR= 1.013, P=0.072; OR=1.017, P=0.032). The correlation between serum total SCFAs and eGFR showed statistical significance (OR= 0.019, P=0.024) unadjusted and a borderline significance (OR= 1.024, P =0.063) when adjusted for Hb and LDL. The decrease in serum acetate and total SCFAs were found of borderline significant correlation in both subgroups (P=0.055, P=0.050).

Conclusion

This study provides evidence that in individuals with DKD, serum and fecal SCFAs levels (fecal level in particular) were lowered, and there was a correlation between lower SCFAs and a worsened renal function.

Introduction

Diabetic kidney disease (DKD) is the most serious complication of diabetic mellitus (DM) and the leading cause of chronic kidney disease (CKD) in the world. A recent study indicated that the prevalence of DM in China was 11.2% (95% confidence interval 10.5% to 11.9%), especially in Han ethnicity [1]. About 35% of patients with type 2 DM (T2DM) would eventually develop DKD, with an increased mortality[2], but the etiology of diabetic kidney disease is yet still unclear.

Recent studies highlighted the involvement of gut microbiome-kidney axis in nephropathy [3, 4]. Tao *et al*. demonstrated that gut microbiota was associated with the development of DKD, and the individuals with DKD could be accurately screened out by testing *g_Escherichia-Shigella* and *g_ Prevotella_9* among the individuals with diabetes [5]. Another study showed that fecal microbiota transplantation could reverse intestinal microbiota dysbiosis and improve renal function in rats with DKD [6]. These suggested that gut microbiota dysbiosis may play an important role in the pathogenesis of DKD.

Besides, studies also indicated that gut microbiota and kidney were interacted *via* microbiome-kidney axis, which also participated in kidney injury process. Being one of the major metabolites of microbiota-mediated fiber fermentation process in the gut, short-chain fatty acids (SCFAs) have attracted considerable interest. SCFAs are a subset of fatty

acids that contain 6 or less carbon molecules and have showed beneficial effects on kidney [4, 7]. SCFAs played a role in biological modulation by attenuating the inflammatory response and reduce mean arterial pressure, *via* inhibiting histone deacetylases (HDACs) and activating G protein receptor 41(GPR41), GPR43, GPR109a and Olfr78[8,9]. However, SCFAs presented markedly varied concentrations in different diseases [10, 11]. The change of fecal and serum SCFAs levels in DKD remains unclear.

In this study, all 90 participants were included from HwaMei Hospital. Fecal and serum samples were measured for SCFAs with gas chromatograph-mass spectrometry (GC-MS). We reported the substantial variations in the levels of fecal and serum SCFAs among normal controls, participants with diabetes and participants with DKD. SCFAs levels in participants with diabetic kidney disease were further analyzed within subgroups by renal function.

Methods

Participants

There were 30 participants with DKD, 30 participants with type 2 diabetes, and 30 normal controls included in HwaMei Hospital, University of Chinese Academy of Science from 1 January 2018 to 31 December 2019. The diagnosis of T2DM were defined by the criterion issued by American Diabetes Association (ADA) in 2017[12]. Diabetic kidney disease can be diagnosed when patients with type 2 diabetes meet any of the following situation: 1)microalbuminuria with an ACR between 30-300mg/g; 2)macroalbuminuria with an ACR>300mg/g. 3) 2 of 3 samples should fall within the microalbuminuric or macroalbuminuric range to confirm clsaaification in the absence of urinary tract infection with 2 additional first-void specimens collected over the next 3 to 6 months.[13]. All participants underwent a medical history screening, a physical examination and body mass index (BMI) was calculated. Lab tests were complete blood count and metabolic panel including albumin, fasting glucose, lipid profile, renal function and urinary albumin creatinine ratio (UACR) . Estimated glomerular filtration rate (eGFR) was calculated with the CKD-EPI$_{Scr}$ formula. Participants in NC group from physical examination center were given tests including metabolic panel, urinalysis, stool test, HBsAg (Hepatitis B surface antigen) and anti-HCV(Hepatitis C antibody). Exclusions include: receiving antibiotics or probiotics within 2 months, gastro-intestinal or systemic diseases known to affect gut bacterial composition, primary or other secondary kidney disease, obesity, liver cirrhosis with/without complications, non-alcoholic fatty liver disease, HBsAg or anti-HCV positive. The clinical parameters are shown in Table 1.The flow diagram is shown in Supplementary Figure 1. The research protocols were conformed to the provisions of the Declaration of Helsinki and were approved by the Ethic Committee of HwaMei Hospital, University of Chinese Academy of Sciences (No.2017-011-01). Informed consent for the study and the publication was obtained from each participant.

Fecal and serum sample collection

Fresh fecal samples were collected and a portion of 200mg was utilized for each test. Blood samples were collected in the fasting status and serum was obtained by centrifugation at 3,500rpm for 5min at 4°C. These samples were then stored at -80°C until usage. One fecal sample and one serum sample in DKD group were later found not usable and were excluded in the study. 30 serum samples in NC group were not collected from physical examination center. Hence, 30 fecal samples in NC group, 30 fecal and serum samples in the diabetes group, 29 fecal and serum samples in the group with DKD were used for data determination.

Fecal and serum sample processing

Each fecal sample of 200mg was mixed with 0.8mL of ultrapure water, crushed with a tissue grinder and then centrifuged at 12,000 rpm for 20 min at 4℃. Each 0.4ml supernatant was mixed with 0.1mL of 50% sulfuric acid (ultrapure water diluted), 0.5ml of ether(containing 50ug /mL of internal standard dimethylvaleric acid) for 1 min, centrifuged at 12,000 rpm for 20 min at 4℃, and then stood for 30 min at 4℃. The supernatant ether layer was filtered through anhydrous sodium sulfate for GC–MS analysis.

Each serum sample (100μL) was mixed with 50μL of 50% sulfuric acid (ultrapure water diluted), 200μL of ether (containing standard dimethylvaleric acid) for 1 min, centrifuged at 12,000 rpm for 20 min at 4℃, and then stood for 30 min at 4℃. The supernatant ether layer was filtered through anhydrous sodium sulfate and the solution later transferred to a glass vial for GC–MS analysis.

Determination of SCFAs using gas chromatograph-mass spectrometry (GC-MS)

The analysis was performed using the GC-MS 7890A-5975C (Agilent Technology, USA). A FFAP capillary column (30m×0.25mm×0.25μm) was used for chromatographic separation, and helium (1 mL/min) was used as the carrier gas. The stepwise chromatographic thermal conditions were as follows: 100°C for 1 min, 5°C/min to 160°C, 40°C/min to 240°C, maintaining for 10min. The mass spectrometer was set to scan mode at m/z 100–300 and selected ion monitoring mode at m/z 60 for acetate, butyrate, iso-valerate , valerate and caproate, maintaining for 4.72min, 7.34min□8.90min, 8.03min and 11.26min respectively, as well as m/z 73 for propionate and iso-butyrate for 5.90min and 6.31min separately.

Statistical analysis

All statistical analyses were performed with SPSS Statistics 19.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 7.0. The results were expressed as means with standard deviation (SD) for normally distributed continuous variables, median values (interquartile ranges) for non-normally distributed continuous variables and frequencies and percentages for categorical variables. ANOVA or Student's t-test for independent samples was used for normally distributed continuous variables. Comparisons of non-normally distributed continuous variables were performed using the Mann-Whitney U-test or Kruskal-Wallis test. For categorical variables, the chi-square test was used. Correlation difference between variables was analysed by Spearman's R coefficient using psych package 1.9.12, and visualized by heatmap in corrplot package 0.84. The association between fecal or serum level with the clinic index was examined via binary logistic regression analysis, based on median level of fecal or serum SCFAs. Covariates with P<0.1 in the univariate regression analysis were chosen for multivariate regression analysis. A P value<0.05 was considered statistically significant.

Results

Baseline characteristics among the three groups

Baseline clinical and biochemical characteristics of all participants in NC group, DM group and DKD group are shown in Table 1. Participants had a mean age of 51.93±8.62 years in NC group (15 males, 15 females), 59.10±8.45 years in DM group (19 males, 11 females) and 61.17±8.09 years in DKD group (24 males, 6 females). The median duration of diabetes in DKD was longer than that in DM group. Patients in DKD group were significantly higher in serum creatinine and urinary albumin creatinine ratio (UACR) and lower in eGFR comparing with patients in DM and/or NC group (P<0.001). Meanwhile DKD group also had a markedly lower hemoglobin and serum albumin comparing with NC group (P<0.001). Among the three groups, the level of total cholesterol, triglyceride, low-density lipoprotein and were

similar without statistical significance. The percentage of participants using metformin had no difference between DM group and DKD group.

Comparisons of fecal and serum SCFAs among the three groups

The acetate, propionate, butyrate, iso-butyrate, valerate, iso-valerate and caproate in stool sample were identified (Figure 1). Notably, the content of acetate in stool was markedly lower in the group with DKD versus diabetes (P=0.003) and NC group (P<0.001). Lower propionate and butyrate levels in DKD group were observed compared with NC group (P<0.05). Correspondingly, fecal total SCFAs presented in the same trend, being lowest in DKD group, 3843.01(2491.81, 5290.88) μg/g, while highest in NC group, reaching 6482.68(4438.91, 8379.59) μg/g (P<0.001). However, the median levels of iso-butyrate, valerate, iso-valerate and caproate were equivalent among the three groups (P>0.05).

Meanwhile, serum SCFAs were also measured in DM and DKD groups (Figure2). We observed a significant difference in serum caproate in DM group[0.65(0.53, 0.79) μmol/L] versus DKD group [0.57(0.47, 0.61) μmol/L] (P<0.05). A strong tendency towards statistical significance was also seen in serum iso-butyrate, valerate and iso-valerate, each was lower in DKD group comparing with DM group(P=0.081, P=0.050, P=0.070, respectively). Apart from this, other SCFAs between DM group and DKD group showered no difference. Unexpectedly, there was no correlation between serum SCFAs and corresponding fecal SCFAs (raw P>0.05)

The correlations between SCFAs and the biochemical indicators

Correlations between the fecal SCFAs and clinical indicators were estimated by Spearman's correlation analysis (Figure 3). As expected, an inverse relationship was observed between blood urea nitrogen and fecal acetate, propionate and butyrate levels(r=-0.22, P=0.03; r=-0.27, P<0.01; r=-0.21, P=0.03, respectively). Meanwhile, UACR was negatively related with fecal acetate (r=-0.38, P<0.01). Interestingly, hemoglobin and serum albumin level showed a positive relationship with fecal acetate, propionate and butyrate (P<0.05). Blood glucose negatively related with fecal acetate and propionate (r=-0.32, P<0.01; r=-0.25, P=0.01, respectively).

We further investigated the correlations between serum SCFAs and biochemical indicators (Figure 4). Unexpectedly, no statistical correlations were found between renal function markers and serum SCFAs, except for a negative correlation between age and acetate level (r=-0.25, P=0.04), positive correlations between total cholesterol, low-density lipoprotein and propionate (r=0.31, P=0.03; r=0.29, P=0.02).

In the univariate regression analysis, fecal acetate and serum acetate were both correlated with eGFR with statistical significance (OR= 1.013, 95%CI (0.999, 1.028), P=0.072; OR=1.017, 95%CI (1.002, 1.034), P=0.032) (Tables 2 and 3). However, in multivariate analysis, acetate in stool(Table 2) or serum(Table 3) showed no correlation with eGFR (P>0.01). Total SCFAs correlated with eGFR in subjects with statistical significance [OR= 1.019, 95%CI (1.002, 1.035), P=0.024] unadjusted while the correlation became borderline significant [OR = 1.024, 95%CI (0.999, 1.050), P =0.063] (Table 4) when adjusted for Hb and LDL. Interestingly, fecal acetate, serum acetate and total SCFAs each related with Hb in subjects with statistical significance [OR = 1.032, 95%CI (1.009, 1.056), P =0.007; OR= 1.026, 95%CI (1.000, 1.052), P =0.049; OR = 1.027, 95%CI (1.002, 1.054), P =0.038].(Tables 2-4)

The subgroup analysis of fecal and serum SCFAs in DKD

To study the fecal and serum SCFAs in patients with various renal function, we categorized the DKD patients into two subgroups according to the eGFR level, the low GFR subgroup (eGFR<60ml/min, n=14) and the high GFR subgroup (eGFR≥60ml/min, n=16). The baseline data of the two subgroups were shown in Supplementary table 1. Age, gender

and BMI between the two groups were matched with no statistical difference (P>0.05). UACR, serum creatinine and blood urea nitrogen were statistical higher (P<0.05) in the low GFR subgroup compared with high GFR subgroup.

There were no differences in fecal SCFAs between the two subgroups (P>0.05). As shown in Supplementary table 2, serum acetate and total SCFAs were lower and with borderline significant in low GFR subgroup versus high GFR subgroup (P=0.055, P=0.050, respectively). However, other SCFAs had no difference between these two subgroups (P>0.05).

Discussion

We are the first to investigate fecal and serum SCFAs simultaneously in individuals with DKD. In this study, fecal acetate, propionate, butyrate and total SCFAs were markedly lower in DKD group. Serum acetate and total SCFAs were also found lower in low eGFR subgroup. Furthermore, fecal and serum acetate seem to be respectively correlated with eGFR in DKD patients. Besides, serum total SCFAs seems to be an independent factor for renal function.

SCFAs are end products of bacterial carbohydrate fermentation, and function as an important energy source and signaling molecules [14]. The concentration of SCFAs varies among different diseases. In DKD mice, there was a significantly decreasing in propionic acid and butyric acid contents in DKD progression [15]. The study conducted by Wang *et al* showed that fecal SCFAs declined in CKD patients, and negatively correlated with the renal function [16]. It was consistent with our study that SCFAs, mainly acetate, propionate and butyrate levels were evidently lower in DKD patients compared to DM and NC groups.

The gut microbiota, yielding SCFAs as the major products, was also believed to involve with DKD. Studies have clearly outlined the changes in microbiota in DKD patients [5, 17], that the richness of gut microbiota and the variation of bacteria population were found different in DKD compared to DM [5] and SCFAs-producing bacteria *Prevotella* declining in DKD patients [5]. We speculated that this reduction of SCFAs-producing bacteria was accompanied by the decrease of yielding SCFAs. Maybe this was the result of the lowest fecal SCFAs levels in DKD.Besides, it may be related with diet, as patients with kidney diseases are required to avoid the intake of fruits, vegetables, and high-fiber diets[16]. So it is warranted to analysis the composition and construction of gut microbiota in newly diagnosed DKD patients in future.

Despite the finding of fecal SCFAs changes, there has not been a defined study on the subsequent serum SCFAs in DKD patients. Our study revealed that the serum acetate was lower in low GFR subgroup than high GFR subgroup with significant difference. This change is postulated to be caused by changes in diet, medication, gastrointestinal microecology and host physiology and pathology. However, we noticed that the main types of SCFAs, including acetate, propionate, butyrate, and valerate did not change significantly in DKD patients versus DM patients, which was unexpected given recent literature identifying a significant decline in SCFA-producing bacteria with advancing kidney disease[17]. Wang *et al* demonstrated that serum acetate and butyrate level was significantly lower in CKD 5 patients than in CKD 1-4 patients [16]. Jadoon *et al* found a significant graded decrease in the concentration of acetate, but the plasma valerate concentration increased in patients with advancing kidney disease than in mild CKD patients [18]. Paradoxically, in streptozotocin (STZ) induced DKD rats, serum acetate levels were markedly elevated compared with controls[6]. The conclusions indicated by our study vary from the above studies , assuming that being associated with the small sample size and the few participants with CKD 5, as well as the low peripheral concentration of SCFAs, which may mitigate the changes[16]. Furthermore, the discrepancies of SCFAs change were possibly due to different etiology of CKD, various severities of the disease, and different animal models [6].Meanwhile, intestinal microecology is known to be complex and each type of bacteria plays a role when the ecology changes. Therefor it is significant to

investigate the types and concentrations of SCFAs in a larger group of DKD patients. Notably, we identified that a significant decline of the level of serum caproate in DKD patients than DM patients in our study. It in in line with with the study that serum caproate concentration decreased in CKD 3 patients compared to non-CKD participants conducted by Wu *et al* [11].

SCFAs diffuse through the intestinal mucosa and enter the bloodstream *via* the portal vein [19, 20]. Samuel *et al* found that the intestinal absorption of SCFA seems to be influenced by the G protein-coupled receptor (GPCR), which are broadly distributed in mammalian organisms [21]. However, serum SCFAs were not in parallel with fecal SCFAs changes in DM and DKD patients in our study. It is assumed that SCFAs measured in circulation may not be utilized in fecal SCFAs excretion, therefore fecal SCFA may be more accurate in revealing SCFAs absorption or production [22]. Several *in vitro* and *in vivo* studies have confirmed significant disruption of the colonic, ileal, jejunal and gastric epithelial tight junction in different models of CKD in rats and in cultured human colonocytes exposed to uremic human plasma [23, 24]. Meanwhile, several observation have provided indirect evidence of increased intestinal permeability in the CKD patients and animals [25, 26]. A human study showed that the participants with lower fecal acetate tended to have higher acetate absorption [22]. However, the transit time of SCFAs in the large intestine does not indicate specific phases of a certain disease. Also, the level of serum SCFAs is influenced by diet manipulations. Herein, we agree that serum SCFAs are effected by many factors and it was necessary to assess both fecal and circulating SCFAs in certain disease to achieve a better understanding of the microbiota change.

Gut microbiota participates in the progression of metabolic diseases *via* its metabolites. Several studies have demonstrated that SCFAs play a protective role in kidney disease. Yang *et al*. revealed that dietary fiber supplement significantly reversed kidney injuries in CKD mice due to increased SCFAs production from microbial fermentation [27]. Andrade-Oliveira *et al*. demonstrated that intraperitoneal injection with SCFAs improved acute kidney injury (AKI) by decreasing inflammatory cytokines and chemokines locally and systemically *via* suppressing NF-κB signaling pathway [28]. In the recent studies, SCFAs played an important effect on multipe aspects of renal physiology, inhibiting inflammation, immunity, and fibrosis, decreasing blood pressure, and adjusting energy metabolism[29].

Protective effects of SCFAs on DKD have also been reported, *via* activation of GPCRs and the inhibition of HDAC activity. Administration of sodium butyrate (NaBu), the major members of SCFAs, ameliorates mesangial matrix expansion, fibrosis and inflammation in the kidneys of STZ-induced diabetic rats [30, 31]. *In vitro* study, NaBu acted as an antioxidant in HG-induced NRK-52E cells and suppressed HG-induced apoptosis of NRK-52E cells through inhibiting HDAC2[32]. In vivo study, dietary fiber protects against DKD through modulation of the gut microbiota, enrichment of SCFA-producing bacteria, and increased SCFA production, so that it reduced expression of genes encoding inflammatory cytokines, chemokines, and fibrosis-promoting proteins in diabetic kidneys *via* GPR43 and GPR109A[33].Recent studies found GPR41 and GPR43 protein expressed in the distal renal tubules and collecting tubules, and found SCFAs lowered TNF-α induced MCP-1 expression by reducing phosphorylation of p38 and JNK in a GPR41/43-dependent manner in human renal cortical epithelial cells(HRCEs)[34]. Iso-butyrate, valerate and iso-valerate, have not been studied as extensively as other SCFAs, and details of the physiological effects are sparse. Previous work has identified these as ligands for GPCR[35], which influence a variety of metabolic, immune and vascular processes[36].

There are some limitations in our cross-section study, consequently we could not demonstrate the causal relationship between fecal, serum SCFAs and the presence of DKD. This monocentric study included a small number of patients in China and dietary assessment was not included in the study design . Prudence need to be taken when trying to extrapolate our data to other populations. Besides, the composition and construction of gut microbiota in participants were not analyzed, therefor the relationship between fecal and serum SCFAs and gut microbiota was not identified.

In conclusion, this study provides evidence for quantitative reduction of gut microbial product - SCFAs （fecal acetate, propionate and butyrate in particular） in DKD patients, demonstrating the association of SCFAs with worse renal function in DKD.

Abbreviations

DKD: diabetic kidney disease; SCFA:short chain fatty acids; HDACs: histone deacetylases; GC-MS: gas chromatograph-mass spectrometry; GPCR: G protein-coupled receptor; HRCEs: human renal cortical epithelial cells

Declarations

Achnowledgements

We sincerely appreciate the participation of each patient in the study. The GC/MS was performed by Metabo-Profile Biotechnology (Shanghai) Co.,Ltd

Authors' contributions

Study conception and design: KC, CZ, QL; Administrative support: KC, QL. Patient education and instruction: KC, CZ, LW, JL, WG, QZ, JZ; Collection and assembly of data: KC, CZ, ZD, LC, LW, JL, WG, QZ, JZ, CX, HL; Data analysis and interpretation: KC, CZ, QL; KC. and CZ.took the lead in writing /wrote the manuscript with input from all authors.Manuscript writing: All authors. Final approval of manuscript: All authors

Funding

This study was supported by the funds from Zhejiang Provincial Natural Science Foundation of China (LY20H05005), Ningbo Public Service Technology Foundation, China (2019C50084) and Medical Scientific Research Foundation of Zhejiang Province, China (2019KY174).

Availability of data and materials

All data generated and/or analyzed during this study are available from the corresponding authors upon reasonable request.

Ethics approval and consent to participate

This study was approved by the ethics committee of HwaMei Hospital, University of Chinese Academy of Sciences and all participants provided written informed consent.

Consent for publication

Consent for publication of clinical data was obtained from all participants

Competing interests

The authors declare that they have no conflicts of interest.

References

1. Li Y, Teng D, Shi X et al, Qin G, Qin Y, Quan H, et al. Prevalence of diabetes recorded in mainland China using 2018 diagnostic criteria from the American Diabetes Association: national cross sectional study. BMJ. 2020;369:m997. doi:10.1136/bmj.m997
2. Demmer RT, Zuk AM, Rosenbaum M, Desvarieux M. Prevalence of diagnosed and undiagnosed type 2 diabetes mellitus among US adolescents: results from the continuous NHANES, 1999–2010. Am J Epidemiol. 2013;178:1106–13. doi:10.1093/aje/kwt088
3. Lu CC, Ma KL, Ruan XZ, Liu BC. Intestinal dysbiosis activates renal renin-angiotensin system contributing to incipient diabetic nephropathy. Int J Med Sci. 2018;15:816–22. doi:10.7150/ijms.25543
4. Andrade-Oliveira V, Amano MT, Correa-Costa M, Castoldi A, Felizardo RJF, Almeida DC, et al. Gut Bacteria Products Prevent AKI Induced by Ischemia-Reperfusion. J Am Soc Nephrol. 2015;26: 1877–88. doi:10.1681/ASN.2014030288
5. Tao S, Li L, Li L, Liu Y, Ren Q, Shi M, et al. Understanding the gut-kidney axis among biopsy-proven diabetic nephropathy, type 2 diabetes mellitus and healthy controls: an analysis of the gut microbiota composition. Acta Diabetol. 2019;56:581–92. doi:10.1007/s00592-019-01316-7.
6. Hu ZB, Lu J, Chen PP, Lu CC, Zhang JX, Li XQ, et al. Dysbiosis of intestinal microbiota mediates tubulointerstitial injury in diabetic nephropathy via the disruption of cholesterol homeostasis. Theranostics. 2020;10:2803–16. doi:10.7150/thno.40571
7. Felizardo RJF, Castoldi A, Andrade-Oliveira V, Câmara NOS. The microbiota and chronic kidney diseases_ a double-edged sword. Clin Transl Immunology. 2016;5:e86. doi:10.1038/cti.2016.36
8. Vinolo MAR, Rodrigues HGR, Nachbar RT, Curi R. Regulation of inflammation by short chain fatty acids. Nutrients. 2011;3:858–76. doi:10.3390/nu3100858
9. Kim S, Goel R, Kumar A, Qi Y, Lobaton G, Hosaka k, et al. Imbalance of gut microbiome and intestinal epithelial barrier dysfunction in patients with high blood pressure. Clin Sci(Lond). 2018;132: 701–18. doi:10.1042/CS20180087
10. Huart J, Leenders J, Taminiau B, Descy J, Saint-Remy A, Daube G, et al. Gut Microbiota and Fecal Levels of Short-Chain Fatty Acids Differ Upon 24-Hour Blood Pressure Levels in Men. Hypertension. 2019;74: 1005–13. doi:10.1161/HYPERTENSIONAHA.118.12588
11. Wu IW, Gao SS, Chou HC, Yang HY, Chang LC, Kuo YL, et al. Integrative metagenomic and metabolomic analyses reveal severity-specific signatures of gut microbiota in chronic kidney disease. Theranostics. 2020;10:5398–411. doi:10.7150/thno.41725
12. American Diabetes Association. Standards of Medical Care in Diabetes–2017 Abridged for Primary Care Providers. Clin Diabetes. 2017;35:5–26. doi:10.2337/cd16–0067
13. KDOQI. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease. Am J Kidney Dis. 2007;49(2 Suppl 2):S12–154. doi: 10.1053/j.ajkd.2006.12.005.
14. Koh A, Vadder FD, Kovatcheva-Datchary P, Bäckhed F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. Cell. 2016;165:1332–45. doi:10.1016/j.cell.2016.05.041
15. Li Y, Su X, Gao Y, Lv C, Gao Z, Liu Y, et al. The potential role of the gut microbiota in modulating renal function in experimental diabetic nephropathy murine models established in same environment. Biochim Biophys Acta Mol Basis Dis. 2020;1866:165764. doi:10.1016/j.bbadis.2020.165764.
16. Wang S, Lv D, Jiang S, Jiang J, Liang M, Hou F, et al. Quantitative reduction in short-chain fatty acids, especially butyrate, contributes to the progression of chronic kidney disease. Clin Sci(Lond). 2019;133:1857–70. doi:10.1042/CS20190171

17. Felizardo RJF, Watanabe IKM, Dardi P, Rossoni LV, Câmara NOS.The interplay among gut microbiota, hypertension and kidney diseases: The role of short-chain fatty acids. Pharmacol Res. 2019;141: 366–77. doi:10.1016/j.phrs.2019.01.019
18. Jadoon A, Mathew AV, Byun J, Gadegbeku CA, Gipson DS, Afshinnia F, et al. Gut Microbial Product Predicts Cardiovascular Risk in Chronic Kidney Disease Patients. Am J Nephrol. 2018;48:269–77. doi:10.1159/000493862
19. Meijers B, Jouret F, Evenepoel P. Linking gut microbiota to cardiovascular disease and hypertension: Lessons from chronic kidney disease. Pharmacol Res. 2018;133:101–7. doi:10.1016/j.phrs.2018.04.023.
20. Pluznick, JL, Protzko RJ, Gevorgyan H, Peterlin Z, Sipos A, Han J. et al. Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. Proc Natl Acad Sci U S A. 2013;110: 4410–5. doi:10.1073/pnas.1215927110
21. Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, et al. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. Proc Natl Acad Sci U S A. 2008;105:16767–72. doi:10.1073/pnas.0808567105
22. Cuesta-Zuluaga J, Muelle NT, Álvarez-Quintero R, Velásquez-Mejía EP, Sierra JA, Corrales-Agudelo V, et al. Higher Fecal Short-Chain Fatty Acid Levels Are Associated with Gut Microbiome Dysbiosis, Obesity, Hypertension and Cardiometabolic Disease Risk Factors. Nutrients. 2018; 11:51.doi:10.3390/nu11010051
23. Vaziri ND, Yuan J, Nazertehrani S, Ni Z, Liu S. Chronic kidney disease causes disruption of gastric and small intestinal epithelial tight junction. Am J Nephrol. 2013;38:99–103. doi:10.1159/000353764.
24. Vaziri ND, Zhao YY, Pahl MV. Altered intestinal microbial flora and impaired epithelial barrier structure and function in CKD: the nature, mechanisms, consequences and potential treatment. Nephrol Dial Transplant. 2016;31:737–46. doi:10.1093/ndt/gfv095.
25. Magnusson M, Magnusson KE, Sundqvist T, Denneberg T. Increased intestinal permeability to differently sized polyethylene glycols in uremic rats: effects of low- and high-protein diets. Nephron. 1990;56:306–11. doi:10.1159/000186158.
26. Magnusson M, Magnusson KE, Sundqvist T, Denneberg T. Impaired intestinal barrier function measured by differently sized polyethylene glycols in patients with chronic renal failure. Gut. 1991;32:754–9. doi:10.1136/gut.32.7.754
27. Yang J, Li Q, Henning SM, Zhong J, Hsu M, Lee R, et al. Effects of Prebiotic Fiber Xylooligosaccharide in Adenine-Induced Nephropathy in Mice. Mol Nutr Food Res 2018;e1800014. doi:10.1002/mnfr.201800014.
28. Andrade-Oliveira V, Amano MT, Correa-Costa M, Castoldi A, Felizardo RJF, Almeida DC, et al. Gut Bacteria Products Prevent AKI Induced by Ischemia-Reperfusion. J Am Soc Nephrol. 2015;26:1877–88. doi:10.1681/ASN.2014030288
29. Li LZ, Tao SB, Ma L, Fu P. Roles of short-chain fatty acids in kidney diseases. Chin Med J (Engl). 2019; 132:1228–32. doi: 10.1097/CM9.0000000000000228.
30. Khan S, Jena G. Sodium butyrate, a HDAC inhibitor ameliorates eNOS, iNOS and TGF-β1-induced fibrogenesis, apoptosis and DNA damage in the kidney of juvenile diabetic rats. Food Chem Toxicol. 2014;73:127–39. doi:10.1016/j.fct.2014.08.010.
31. Dong W, Jia Y, Liu X, Zhang H, Li T, Huang W, et al. Sodium butyrate activates NRF2 to ameliorate diabetic nephropathy possibly via inhibition of HDAC. J Endocrinol. 2017;232:71–83. doi:10.1530/JOE-16-0322.
32. Du Y, Tang G, Yuan W. Suppression of HDAC2 by sodium butyrate alleviates apoptosis of kidney cells in db/db mice and HGinduced NRK52E cells. Int J Mol Med. 2020;45:210–22. doi:10.3892/ijmm.2019.4397

33. Li YJ, Chen X, Kwan TK, Loh YW, Singer J, Liu Y, et al. Dietary Fiber Protects against Diabetic Nephropathy through Short-Chain Fatty Acid-Mediated Activation of G Protein-Coupled Receptors GPR43 and GPR109A. J Am Soc Nephrol. 2020;31:1267–81. doi:10.1681/ASN.2019101029
34. Kobayashi M, Mikami D, Kimura H, Kamiyama K, Morikawa Y, Yokoi S, et al. Short-chain fatty acids, GPR41 and GPR43 ligands, inhibit TNF-α-induced MCP–1 expression by modulating p38 and JNK signaling pathways in human renal cortical epithelial cells. Biochem Biophys Res Commun. 2017;486:499–505. doi: 10.1016/j.bbrc.2017.03.071.
35. Poul EL, Loison C, Struyf S, Springael JY, Lannoy V, Decobecq ME, et al. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. J Biol Chem. 2003;278:25481–9. doi:10.1074/jbc.M301403200.
36. Mirmonsef P, Zariffard MR, Gilbert D, Makinde H, Landay AL, Spear GT. Short-chain fatty acids induce pro-inflammatory cytokine production alone and in combination with toll-like receptor ligands. Am J Reprod Immunol. 2012;67:391–400. doi:10.1111/j.1600-0897.2011.01089.x.

Tables

Table 1: Baseline clinical characteristics of participants

characteristics	NC(n=30)	DM(n=30)	DN(n=30)	*P* value
Age(years)	51.93±8.62	59.10±8.45[a]	61.17±8.09[b]	<0.001**
Gender, male(n, %)	15(50%)	19(63.3%)	24(80%)	0.052
Duration of the disease(years)	–	8.22±7.41	12.43±6.24	0.022*
Body mass index(BMI, Kg/m^2)	23.51±2.33	24.70±5.96	25.29±3.68	0.275
Hb(g/L)	142.50(133.75,155.00)	138.50(128.00,153.50)	115.50(96.00,135.75)[b,c]	<0.001**
CRP(mg/L)	–	3.15±3.40	1.89±2.30	0.160
Glucose(mmol/L)	5.16(4.83,5.52)	5.79(5.06,8.34)[a]	6.11(5.15,7.42)[b]	0.001**
HbA1c(mmol /mol)	–	70±2	59±2	0.080
HbA1c(%)	–	8.56±1.96	7.54±1.99	0.080
TC(mmol/L)	4.71(4.27,5.10)	4.35(3.34, 4.96)	4.71(3.38, 5.73)	0.199
TG(mmol/L)	1.24±0.75	1.87±2.21	1.96±1.60	0.181
HDL (mmol/L)	1.46±0.31	1.08±0.25[a]	1.12±0.37[b]	<0.001**
LDL(mmol/L)	2.77(2.35,3.15)	2.71(2.16,3.31)	2.82(1.84,3.09)	0.863
Alb(g/L)	47.11±5.97	42.14±3.79 [a]	38.43±5.68 [b]	<0.001**
BUN(mmol/L)	4.58(4.23,5.70)	5.29(4.61,7.09)	8.77(5.53,16.85) [b,c]	<0.001**
UA(μmol/L)	315.44±79.69	331.32±76.69	378.31±125.96[b]	0.039*
Creatinine(μmol/L)	61.30(52.50,73.05)	54.55(49.25,67.85)	107.10(63.68,266.63) [b,c]	<0.001**
eGFR(ml/min/1.73m^2)	102.67(98.56,110.81)	102.29(96.72,110.41)	64.60(18.03,95.99) [b,c]	<0.001**
UACR(mg/g)	–	5.30(2.30, 22.00)	789.55(354.43, 2097.70) [c]	<0.001**
Metformin(n, %)	–	17(56.7%)	16(53.3%)	0.795

Abbreviation: NC, normal controls; DM, diabetic mellitus; DN, diabetic nephropathy; Hb, hemoglobin; CRP, C-reactive protein; HbA1c, hemoglobin A1c;TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Alb, albumin; BUN, blood urea nitrogen; UA, uric acid; eGFR, estimated glomerular filtration rate;UACR, urine albumin creatinine ratio.

*P<0.05, **P<0.01

[a] P<0.05 DM compared to NC

[b] P<0.05 DN compared to NC

[c] *P*<0.05 DN compared to DM

Table 2: Univariate and multivariate associates of fecal acetate in participants

Variables	Fecal acetate					
	Univariable			Multivariate		
	OR	95%CI	P value	OR	95%CI	P value
Age (year)	0.966	0.922,1.013	0.153			
Gender	0.966	0.406,2.295	0.937			
Body mass index	0.915	0.801,1.046	0.192			
Hb	1.032	(1.009,1.056)	0.007**	1.041	(1.001,1.083)	0.046*
CRP	1.182	(0.953,1.466)	0.127			
Glucose	0.710	(0.531,0.951)	0.022*	0.705	(0.518,0.959)	0.026*
HbA1c	0.985	(0.956,1.014)	0.309			
TC	0.895	(0.591,1.355)	0.601			
TG	0.740	(0.491,1.118)	0.153			
HDL	6.016	(1.498,24.163)	0.011*	6.180	(1.288,29.642)	0.023*
LDL	1.185	(0.677,2.076)	0.552			
Alb	1.141	(1.036,1.257)	0.007**	1.027	(0.918,1.148)	0.643
BUN	0.947	(0.873,1.028)	0.193			
UA	1.001	(0.996,1.005)	0.771			
Creatinine	0.998	0.995,1.002	0.366			
eGFR	1.013	(0.999,1.028)	0.072	1.002	(0.979,1.025)	0.875
UACR	1.000	(0.999,1.000)	0.154			

Abbreviation: Hb, hemoglobin; CRP, C-reactive protein; HbA1c, hemoglobin A1c;TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Alb, albumin; BUN, blood urea nitrogen; UA, uric acid; eGFR, estimated glomerular filtration rate; UACR, urine albumin creatinine ratio.

Table 3: Univariate and multivariate associates of serum acetate in participants

Variables	Serum acetate					
	Univariable			Multivariate		
	OR	95%CI	P value	β	95%CI	P value
Age (year)	0.949	(0.888,1.014)	0.120			
Gender	1.571	(0.503,4.914)	0.437			
Body mass index	1.085	(0.942,1.249)	0.258			
Hb	1.026	(1.000,1.052)	0.049*	1.012	(0.978, 1.047)	0.506
CRP	1.128	(0.896,1.421)	0.306			
Glucose	0.848	(0.696,1.032)	0.100			
HbA1c	0.987	(0.742,1.311)	0.926			
TC	1.112	(0.717,1.725)	0.636			
TG	1.038	(0.790,1.363)	0.790			
HDL	0.702	(0.131,3.767)	0.680			
LDL	1.520	(0.808,2.858)	0.194			
Alb	1.064	(0.959,1.180)	0.244			
BUN	0.898	(0.809,0.996)	0.042*			
UA	0.998	(0.993,1.003)	0.401			
Creatinne	0.995	(0.991,1.000)	0.070			
eGFR	1.017	(1.002,1.034)	0.032*	1.013	(0.991, 1.034)	0.246
UACR	1.000	(0.999,1.000)	0.317			

Abbreviation: Hb, hemoglobin; CRP, C-reactive protein; HbA1c, hemoglobin A1c;TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Alb, albumin; BUN, blood urea nitrogen; UA, uric acid; eGFR, estimated glomerular filtration rate; UACR, urine albumin creatinine ratio.

Table 4: Univariate and multivariate associates of serum total SCFAs in participants

Variables	Serum total SCFAs					
	Univariable			Multivariate		
	OR	95%CI	P value	β	95%CI	P value
Age (year)	0.948	(0.887,1.013)	0.113			
Gender	1.571	(0.503,4.914)	0.437			
Body mass index	1.060	(0.934,1.202)	0.365			
Hb	1.027	(1.002,1.054)	0.038*	0.996	(0.958,1.034)	0.817
CRP	1.118	(0.891,1.403)	0.336			
Glucose	0.861	(0.713,1.039)	0.119			
HbA1c	0.997	(0.750,1.326)	0.984			
TC	1.245	(0.795,1.949)	0.338			
TG	1.033	(0.787,1.356)	0.812			
HDL	0.784	(0.147,4.170)	0.775			
LDL	1.895	(0.970,3.702)	0.061	2.381	(1.068,5.305)	0.034
Alb	1.090	(0.979,1.214)	0.115			
BUN	0.885	(0.792,0.989)	0.031*			
UA	0.998	(0.993,1.003)	0.479			
Creatinne	0.995	(0.990,1.000)	0.066			
eGFR	1.019	(1.002,1.035)	0.024*	1.024	(0.999,1.050)	0.063
UACR	1.000	(0.999,1.000)	0.355			

Abbreviation: Hb, hemoglobin; CRP, C-reactive protein; HbA1c, hemoglobin A1c;TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Alb, albumin; BUN, blood urea nitrogen; UA, uric acid; eGFR, estimated glomerular filtration rate;UACR, urine albumin creatinine ratio.

Figures

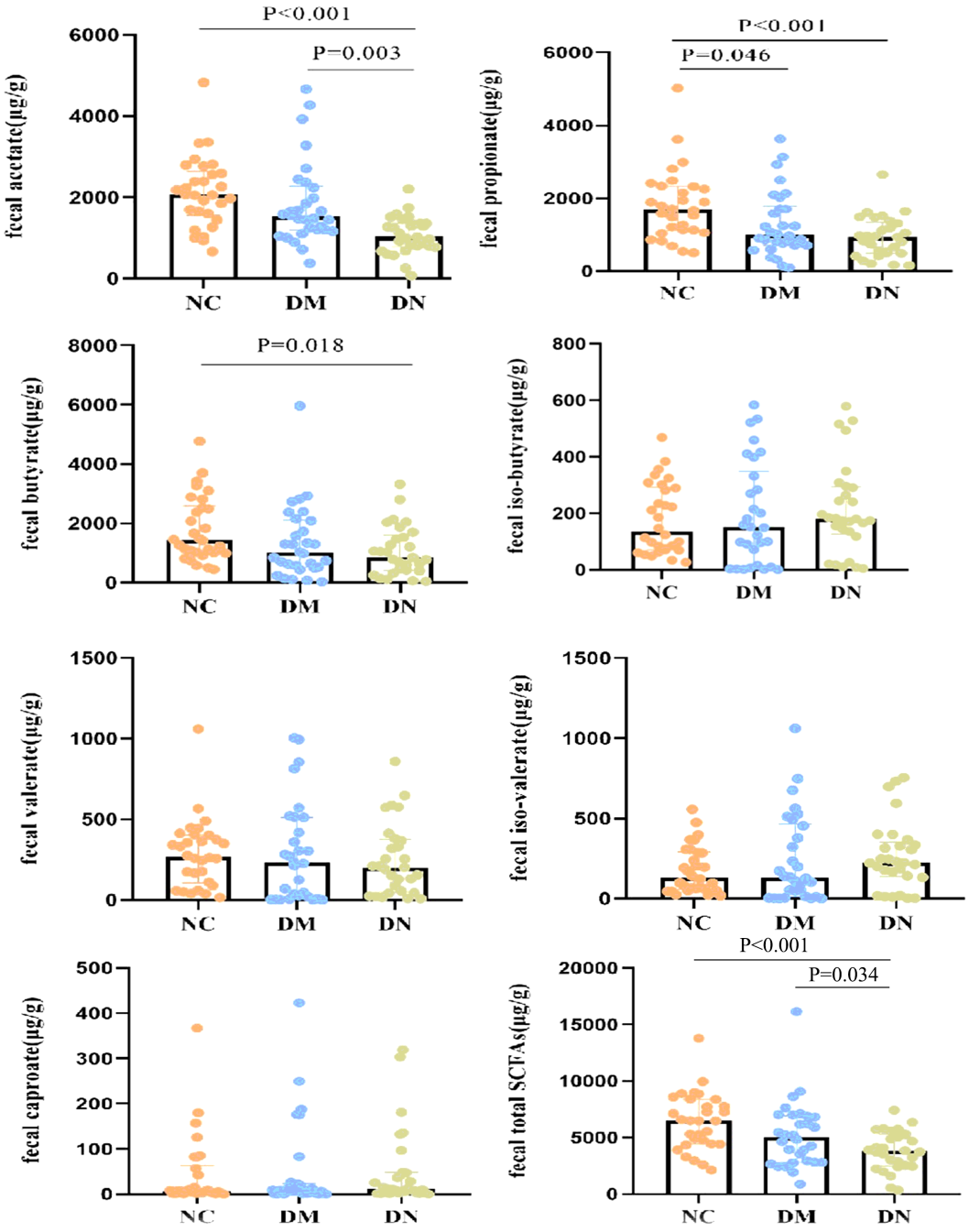

Figure 1

The acetate, propionate, butyrate, iso-butyrate, valerate, iso-valerate and caproate in stool sample were identified (Figure 1).

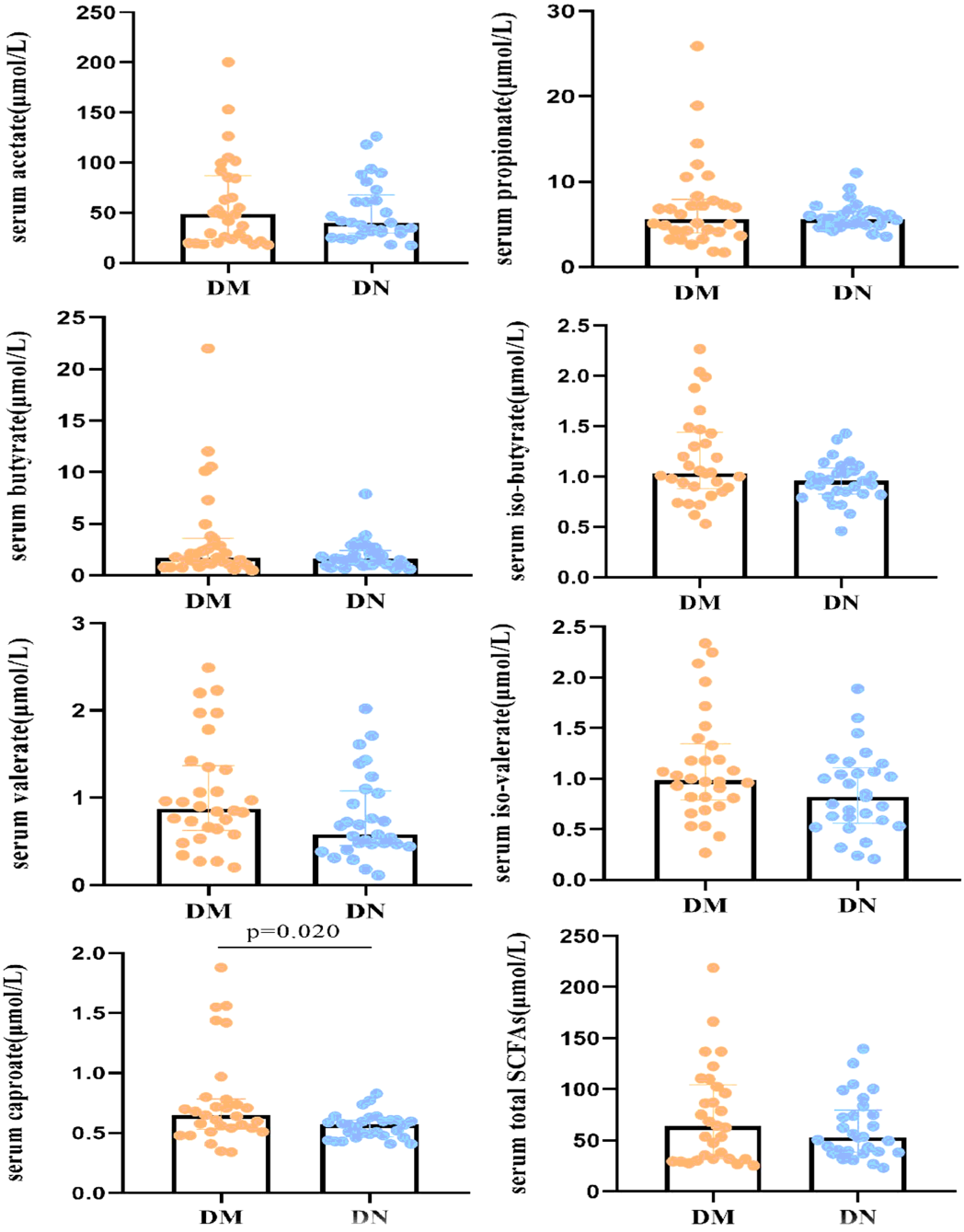

Figure 2

Meanwhile, serum SCFAs were also measured in DM and DKD groups (Figure2).

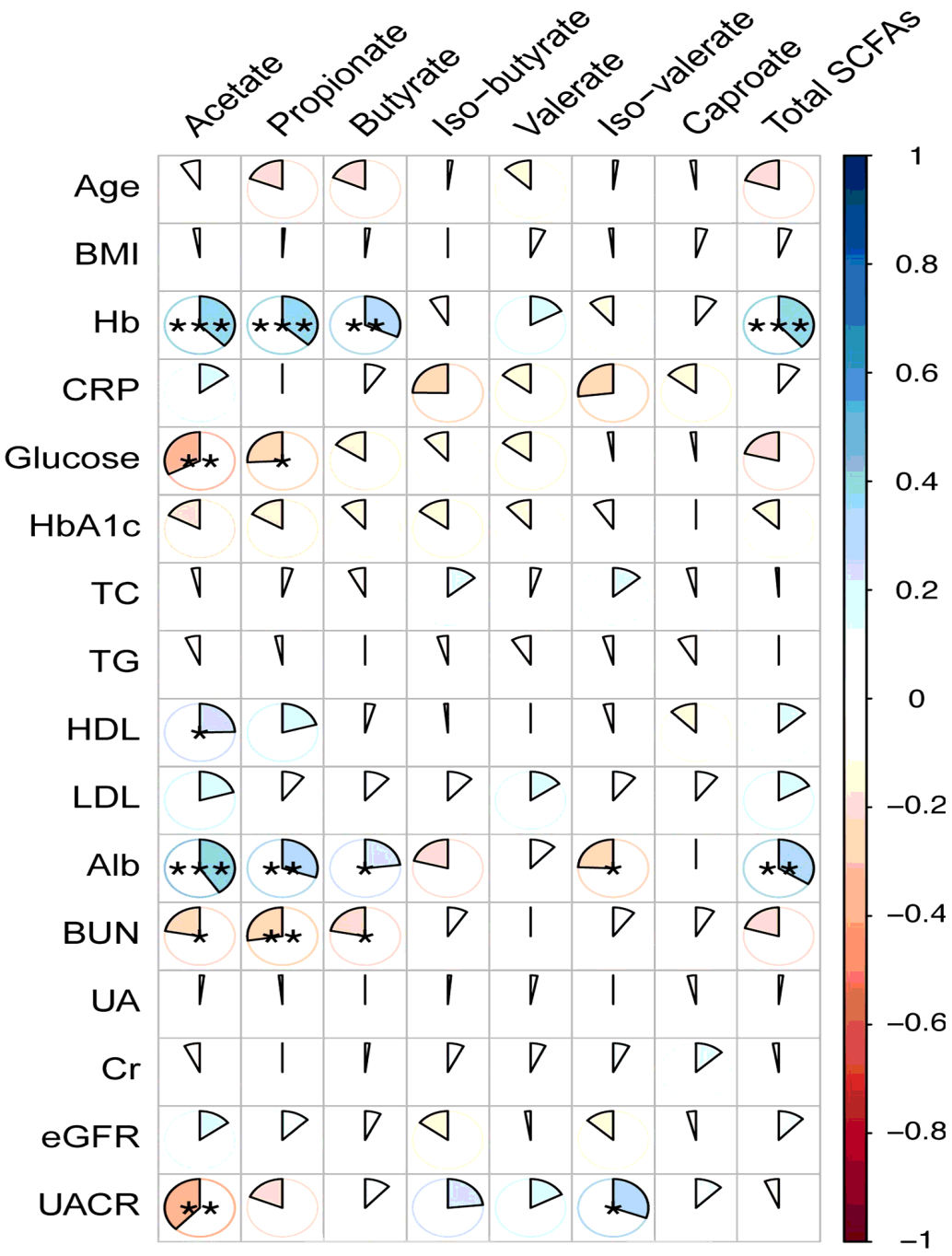

Figure 3

Correlations between the fecal SCFAs and clinical indicators were estimated by Spearman's correlation analysis (Figure 3).

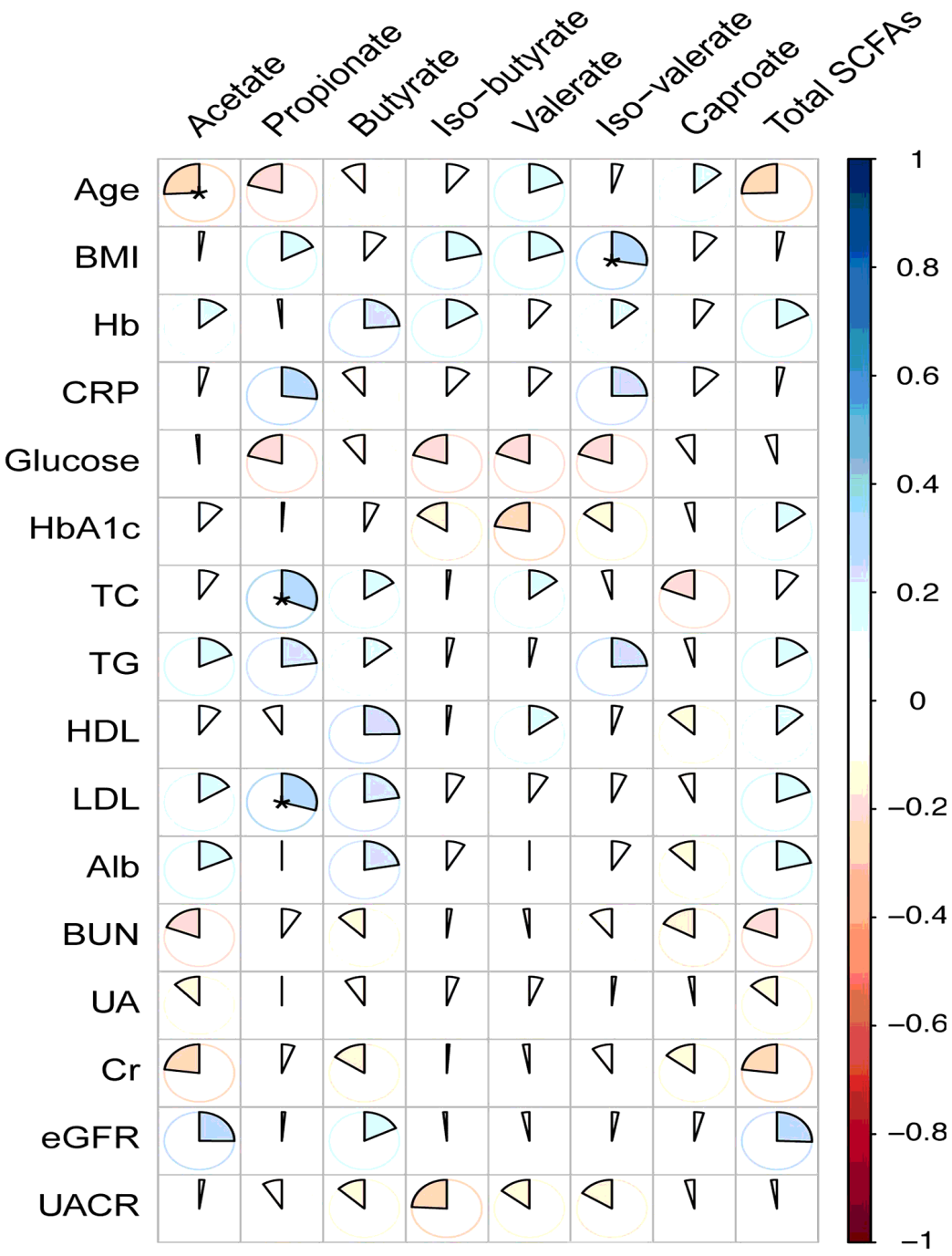

Figure 4

We further investigated the correlations between serum SCFAs and biochemical indicators (Figure 4).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- strobestatement.doc
- supplementary1.doc
- supplementary2.doc

- supplementaryfigure1.tif