Moderation effects of food intake on the relationship between urinary microbiota and urinary interleukin-8 in female type 2 diabetic patients

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Background. Our previous study demonstrated that the composition of the urinary microbiota in female patients with type 2 diabetes mellitus (T2DM) was correlated with the concentration of urinary interleukin (IL)-8. As the composition of urine is mainly determined by diet, diet might mediate the correlation. **Methods.** Seventy female T2DM patients and 70 healthy controls (HCs) were recruited. Midstream urine was used for the urine specimens. Urinary IL-8 was determined by enzyme-linked immunosorbent assay. A Chinese Food Frequency Questionnaire was used to collect food intake data. The independent variables in the hierarchical regression analysis were the relative abundances of the bacterial genera and species that were significantly different between the T2DM and HCs and between the T2DM patients with and without detectable urinary IL-8, and the bacterial genera associated with IL-8 concentration in the multiple regression model reported in our previous research. IL-8 concentration was the dependent variable, and nutrient intakes were moderator variables. **Results.** Fiber and vitamin B3 and E intake exerted enhancing effects, and water intake exerted a buffering effect, on the positive relationship between the relative abundance of *Ruminococcus* and IL-8 concentration (p < 0.05). Cholesterol and magnesium intake exerted enhancing effects on the positive relationship between the relative abundance of *Comamonas* and IL-8 concentration (p < 0.05). **Conclusion.** Modulating T2DM patients' dietary patterns may prevent bladder inflammation.

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2 **interleukin-8 in female type 2 diabetic patients**

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Abstract

Background. Our previous study demonstrated that the composition of the urinary microbiota in female patients with type 2 diabetes mellitus (T2DM) was correlated with the concentration of urinary interleukin (IL)-8. As the composition of urine is mainly determined by diet, diet might mediate the correlation.

Methods. Seventy female T2DM patients and 70 healthy controls (HCs) were recruited. Midstream urine was used for the urine specimens. Urinary IL-8 was determined by enzyme-linked immunosorbent assay. A Chinese Food Frequency Questionnaire was used to collect food intake data. The independent variables in the hierarchical regression analysis were the relative abundances of the bacterial genera and species that were significantly different between the T2DM and HCs and between the T2DM patients with and without detectable urinary IL-8, and the bacterial genera associated with IL-8 concentration in the multiple regression model reported in our previous research. IL-8 concentration was the dependent variable, and nutrient intakes were moderator variables.

Results. Fiber and vitamin B3 and E intake exerted enhancing effects, and water intake exerted a buffering effect, on the positive relationship between the relative abundance of *Ruminococcus* and IL-8 concentration ($p < 0.05$). Cholesterol and magnesium intake exerted enhancing effects on the positive relationship between the relative abundance of *Comamonas* and IL-8 concentration ($p < 0.05$).

Conclusion. Modulating T2DM patients' dietary patterns may prevent bladder inflammation.

Introduction

Diabetes mellitus (DM) is a severe chronic disease that is recognized as a global public health problem. In recent decades, the prevalence of DM has increased substantially. Globally, about 1 in 11 adults have DM (90% have type 2 diabetes mellitus (T2DM)) (Zheng et al., 2018). Infections were more common prevalent with T2DM than among respondents without diabetes (Carey et al., 2018), especially urinary tract infection (UTI) (Wilke et al., 2015). UTI is of major concern, and many studies have demonstrated an increased prevalence of UTI in T2DM patients (Wilke et al., 2015; Hirji et al., 2012; Nichols et al., 2017), especially in female patients (Hirji et al., 2012). Traditionally, UTI has been thought to be caused by bacterial invasion of the patient's bladder. However, studies on the urinary microbiota have caused this concept to be questioned over the past decade, as DNA sequencing and expanded quantitative urine culture technique have shown that bacteria live in almost all individuals' bladders, both UTI patients and healthy subjects (Price et al., 2016; Thomas-White et al., 2018). Therefore, the presence of bacteria in the bladder of UTI patients may just indicate an altered bacterial profile compared to that of healthy individuals rather than the invasion of new bacteria.

An increasing body of evidence suggests that T2DM is associated with profound gut dysbiosis (Tai et al., 2015; Wang et al., 2017). Food intake patterns can modulate the dysbiosis, which in turn impacts metabolic health (David et al., 2014; Simpson & Campbell, 2015). The kidneys are primary excretory

69 organs, and are responsible for the elimination of waste metabolites. Food intake patterns determine the

70 metabolites in urine, which may affect the bacterial profile in the bladder (Shields-Cutler et al., 2015).

71   It has been reported that interleukin (IL)-8 can function as a diagnostic biomarker for UTI, with a

72 sensitivity of 93% and a specificity of 90%, which is much greater than the sensitivity and specificity of

73 urine culturing (Rao et al., 2001). Similarly, another study demonstrated that urinary IL-8 could be

74 considered as a surrogate marker for the rapid diagnosis of bacteriuria (Zaki, 2008). Therefore, we

75 detected the urinary IL-8 concentration in our previous research, and found that 46 out of 70 subjects had

76 detectable concentrations of urinary IL-8 (Ling et al., 2017).

77   Our previous research demonstrated that T2DM patients had lower bacterial diversity in urinary

78 microbiome than healthy controls (HCs). There were significant differences in the relative abundance of

79 multiple urinary bacterial genera between T2DM patients and HCs, including *Lactobacillus*, *Prevotella*,

80 and *Pseudomonas* (Liu et al., 2017). Subgroup analysis showed that T2DM patients with detectable

81 concentrations of urinary IL-8 (WIL8) had a different urinary bacterial profile from those with no

82 detectable concentrations of urinary IL-8 (NIL8); indeed, the WIL8 group had notable elevations and

83 reductions in several bacterial genera and species compared to the NIL8 group. Moreover, subjects in the

84 T2DM group with higher or lower relative abundance of specific urinary bacteria genera, relative to HCs,

85 had different concentrations of IL-8. Additionally, several urinary genera were significantly associated

86 with the urinary IL-8 concentration in T2DM patients, including *Ruminococcus*, *Anaerotruncus*, and

87 *Lactobacillus*. Moreover, the WIL8 group had significantly higher urinary concentrations of nitrites and

88 leukocyte esterase than the NIL8 group (Ling et al., 2017).

89   As stated above, the urinary microbiome profile in T2DM females is different from healthy controls,

90 and the bacterial dysbiois is related to the expression level of IL-8. Studies on gut microbiome have

91 shown that diet regulate the composition of microbiome and inflammation (Tilg & Moschen, 2015). On

92 one hand, gut microbiome can rapidly respond to altered diet. Walker AW et al. demonstrated that

93 increassed intake of resistant starch can substantially alter the species composition of the colonic

94 microbiome (Walker et al., 2011). David LA et al. reported that short-term consumption of diets

95 composed entirely of animal or plant products resulted in microbial community structure (David et al.,

96 2014). On the other hand, studies have shown that intestinal dysbiosis led to an abnormal adaptive

97 immune response that increased inflammatory gut diseases (Seksik et al., 2003). However, to date, no

98 studies have shown whether the relationship between urinary microbiome and IL-8 level is regulated by

99 nutrients intake. Therefore, our present study aimed to analyze the regulating role of nutrient intake on the

100 correlation between the urinary microbiome and IL-8 using moderation effect model. If nutrients intake

101 has a moderating role, clinicians can precisely adjust patient's dietary composition based on the

102 correlation between urinary microbiome and IL-8 concentration.

Materials & Methods

Recruitment of subjects

Briefly, a case-control design was used in our previous study, in which female T2DM patients were individually matched to HCs according to age, marital status, and menstrual status. The same data were used in the present study. The participants were recruited from the First Affiliated Hospital, School of Medicine, Zhejiang University, from July 2, 2015 to January 2, 2016. For every 10 T2DM patients recruited, 10 healthy controls who matched the patients' age, marriage and menstrual status were recruited. Subjects with the following attributes were excluded: UTI in the previous month; use of antibiotics, probiotics, prebiotics, or synbiotics in the previous 3 months; inability to complete the questionnaire; menstruation; urinary incontinence; known anatomic urinary tract abnormalities (e.g., cystoceles, hydronephrosis, renal atrophy, or neurogenic bladder); or urinary catheter use. The Ethics Committee of the First Affiliated Hospital, School of Medicine, Zhejiang University, approved the study (ref. 295). Written informed consent was obtained from each patient before enrollment.

Urine specimen collection, DNA isolation and sequencing, and IL-8 detection

A self-designed midstream urine collection technique, which was reported in our previous study, was used to collect the first urine of the day (Liu et al., 2017). Bacterial DNA was isolated using magnetic beads, and the 16S rRNA gene V3-V4 regions of microbial genomic DNA were amplified by PCR (forward primer, 5′-ACTCCTACGGGAGGCAGCAG-3′; reverse primer, 5′-GGACTACHVGGGTWTCTAAT-3′). Amplicons were then normalized, pooled, and sequenced using an Illumina MiSeq desktop sequencer (2×300 bp paired-end run). Sequence read processing was performed using QIIME (version 1.9.0), which included quality trimming and demultiplexing. Operational taxonomic unit (OTU) selection, using Usearch (version 1.11.1), included dereplication (--derep_fullength), clustering (--cluster_fast, --id 0.97), and chimera detection (--uchime_ref). Taxonomic assignment for individual datasets was conducted using Greengene (version 13.8). The output file was further analyzed using Statistical Analysis of Metagenomic Profiles software (version 2.1.3). Urinary IL-8 concentrations were determined using enzyme-linked immunosorbent assay (ELISA) kits (RayBiotech, Inc., Norcross, GA, USA).

Food intake assessment and nutrient intake calculations

The influence of diet on the human microbiota is long term, so almost all previous studies on the relationship between the human microbiota and nutrition used the Food Frequency Questionnaire (FFQ) (Mandal et al., 2016; Ribeiro et al., 2017; Singh et al., 2017), which requires participants to recall food intake in the previous year. Therefore, a Chinese version of the FFQ was used to assess food intake (Zhao et al., 2010). Prior to the present study, we conducted a pre-experimental study. We evaluated the reproducibility of the FFQ (Chavarro et al., 2009), in which 10 volunteers were invited to filled the FFQ

137   for twice, e. g. FFQ_1 and FFQ_2. The interval time between the two interviews was two weeks. FFQ_1 and

138   FFQ_2. Then we assessed the correlation coefficient of nutrients intake of energy, protein, fat and

139   carbohydrate, and found they were significantly correlated in FFQ_1 and FFQ_2 ($p < 0.05$; data was not

140   shown) indicating that the reproducibility was good. In the meantime, we conducted a qualitative

141   interview. Based on their suggestions, a face-to-face interview was carried out in each participant's

142   kitchen, where the sizes of the participant's dinnerware items were evaluated. The participants were asked

143   to recall their food and water intake patterns over the previous year (up to the day of urine sample

144   collection). Before the interviews, we visited local supermarkets to convert the quantities of certain foods

145   to weights, as most participants were able to remember the amount, but not the weight, of the food they

146   ate. Subsequently, we separated the elements of the reported food intake into large, moderate, and small

147   portion sizes. Data on the intake of Chinese herbs and supplements were also collected. Water intake

148   included drinking water and the water content of foods. The mean duration for assessing each patient's

149   food intake data was 90 min. MATLAB software (version 7.0) was used to convert the amount of food

150   intake into daily nutrient intake.

151   **Statistical analysis**

152   Statistical analysis was performed using SPSS software (version 21.0). For continuous variables,

153   independent t-tests were applied. For categorical variables, either Pearson's chi-square tests or Fisher's

154   exact tests were used depending on assumption validity. A moderation effect can be enhancing, buffering

155   or antagonistic (Fairchild & MacKinnon, 2009). Therefore, hierarchical regression analysis was used to

156   analyze the moderating effects of nutrient intake on the relationships between the urinary microbiota and

157   IL-8 concentration. To establish hierarchical regression models that would be likely to be true

158   representations of real relationships, the independent variables selected were the bacterial genera and

159   species that exhibited differences in relative abundances between the T2DM and HC groups and between

160   the WIL8 and NIL8 groups of T2DM patients, and also the bacterial genera that contributed to the urinary

161   IL-8 concentration in the T2DM cohort (according to our previous research). The moderator variables

162   were nutrient intakes; the dependent variable was urinary IL-8 concentration; and the control variables

163   were fasting blood glucose, urine glucose, age, menstrual status, and body mass index (Liu et al., 2017;

164   Ling et al., 2017). Before conducting hierarchical regression analysis, Pearson's correlation analysis was

165   used to determine whether the independent and dependent variables were significantly correlated. The

166   independent variables that were significantly correlated with the dependent variable were then entered

167   into hierarchical regression models. The hierarchical regression analysis featured three-steps. First, we

168   entered age, body mass index, fasting blood glucose, urine glucose, and menstrual status as control

169   variables, as our previous study showed that these factors affected the urinary microbiota. Second, we

170   entered other independent variables, such as the relative abundances of bacteria and nutrient intakes.

171   Third, we entered the "bacteria × nutrient" interaction terms. The regression effects were then analyzed.

172   All tests of significance were two-sided, and $p < 0.05$ was considered significant. After obtaining β values

173   for the control variables, main effects, and interaction effects, ModGraph-1 software was used to calculate

174   cell means to display moderation effects in a graphical manner. ModGraph-1 software calculated the

175   effects of high, medium, and low nutrient intake on the relationships between the relative abundance of

176   bacteria and IL-8 concentration.

Results

178   **Patient characteristics, DNA sequencing, and IL-8**

179   Seventy T2DM patients were recruited. Their characteristics are shown in Table 1. A total of

180   3,981,519 reads were obtained, accounting for 76.93% of the valid reads. The mean read length was 438

181   bp (range, 423–486 bp). Good's coverage estimator was 98.00%. Sequence data from this study are

182   deposited in the GenBank Sequence Read Archive (accession no. SRP 087709). The mean urinary IL-8

183   concentration was 42.26±64.66 pg/mL (Liu et al., 2017; Ling et al., 2017).

184   **Several nutrients exhibited moderation effects on the associations between the urinary**

185   **microbiota and urinary IL-8 concentrations**

186   In our previous studies, the relative abundance of 33 bacterial genera-level and 9 bacterial species-

187   level OTUs showed significant differences between T2DM patients and HCs (Figures S1 and S2), 21

188   bacterial genera-level and 10 bacterial species-level OTUs showed significant differences between the

189   WIL8 and NIL8 T2DM subgroups (Figures S3 and S4), and 18 bacterial genera were predictors of urinary

190   IL-8 in a stepwise regression analysis (Table S1). In addition, we identified T2DM patients with

191   significant differences in their urinary microbiota compared with HCs, grouping them according to the

192   bacterial genera present in their urine samples ("≥ HCs" or "< HCs"). We then analyzed the urinary IL-8

193   concentrations for the T2DM patients with significantly high or low abundance of a given genus, relative

194   to the HCs. The IL-8 concentration was low in T2DM patients with high *Acinetobacter*, *Microbacterium*

195   and *Megamonas* abundance, but high in T2DM patients with high *Pseudomonas* and *Klebsiella*

196   abundance (Figure S5) (Liu et al., 2017; Ling et al., 2017). These bacterial genera were included in the

197   hierarchical regression analysis (Table S2).

198   The relative abundances of *Ruminococcus*, *Comamonas*, *Cytophaga*, *Providencia*, *Anaerotruncus*,

199   *Giesbergeria*, *Meiothermus* and *Luteibacter* were correlated with increased urinary IL-8 concentrations,

200   whereas *Cloacibacterium* was correlated with decreased IL-8 (Table S3). When these bacteria were used

201   as independent variables, urinary IL-8 was used as the dependent variable, and nutrient intake variables

202   were used as moderating variables (Table 2), hierarchical regression analysis showed that several

203   nutrients moderated the correlation between the urinary microbiota and IL-8.

204   The relative abundance of *Ruminococcus* and water intake had significant effects on urinary IL-8
205   concentration (Table S4). The "*Ruminococcus* × Water" interaction effect was also significant, and it
206   explained 39.00% of the variance in IL-8 concentration. The strongest negative association between the
207   relative abundance of *Ruminococcus* and IL-8 concentration occurred in patients who reported a high
208   water intake, while the weakest negative association occurred for patients who reported a low water
209   intake (Figure 1A). This suggested that water intake buffered the relationship between the relative
210   abundance of *Ruminococcus* and IL-8 concentration.

211   The relative abundance of *Ruminococcus* and fiber intake had significant effects on urinary IL-8
212   concentration (Table S5). The "*Ruminococcus* × Fiber" interaction effect was also significant, and it
213   explained 41.70% of the variance in IL-8 concentration. In addition, the strongest positive association
214   between the relative abundance of *Ruminococcus* and IL-8 concentration occurred in patients who
215   reported a high fiber intake, whereas the weakest association occurred in those who reported a low fiber
216   intake (Figure 1B). This indicated that fiber intake enhanced the positive correlation between the relative
217   abundance of *Ruminococcus* and IL-8 concentration.

218   In addition, the relative abundance of *Ruminococcus* and vitamin B3 intake had significant effects on
219   urinary IL-8. The "*Ruminococcus* × Vitamin B3" interaction effect was also significant, and it explained
220   32.00% of the variance in IL-8 concentration (Table S6). The strongest positive association between the
221   relative abundance of *Ruminococcus* and IL-8 concentration occurred in patients who reported a high
222   vitamin B3 intake, whereas the weakest association occurred in patients who reported a low vitamin B3
223   intake. This indicated that vitamin B3 intake enhanced the positive correlation between the relative
224   abundance of *Ruminococcus* and IL-8 concentration (Figure 1C).

225   The relative abundance of *Ruminococcus* and vitamin E intake also had significant effects on urinary
226   IL-8. The "*Ruminococcus* × Vitamin E" interaction effect was also significant, and it explained 34.40% of
227   the variance in IL-8 concentration (Table S7). Surprisingly, the strongest positive association between the
228   relative abundance of *Ruminococcus* and IL-8 concentration occurred in patients who reported a high
229   vitamin E intake, the weakest association occurred in patients who reported a medium vitamin E intake,
230   and a negative association was apparent in patients who reported a low vitamin E intake (Figure 1D). This
231   suggests that not only did high vitamin E intake enhance the relationship between the relative abundance
232   of *Ruminococcus* and IL-8 concentration, but also that the amount of vitamin E intake moderated this
233   relationship in a highly sensitive manner.

234   Vitamin C intake had a significant main effect in the regression model involving the relationship
235   between the relative abundance of *Ruminococcus* and IL-8 concentrations, but there was no
236   "*Ruminococcus* × Vitamin C" interaction effect (Table S8).

237   The relative abundance of *Comamonas* and cholesterol intake had significant effects on urinary IL-8.

238   The "*Comamonas* × Cholesterol" interaction effect was also significant, and it explained 68.00% of the

239   variance in IL-8 concentration (Table S9). The strongest positive association between the relative

240   abundance of *Comamonas* and IL-8 concentration occurred in patients who reported a high cholesterol

241   intake, and the weakest association occurred in patients who reported a low cholesterol intake (Figure

242   2A). This suggested that cholesterol intake enhanced the relationship between the relative abundance of

243   *Comamonas* and IL-8.

244   Similarly, the relative abundance of *Comamonas* and magnesium intake had significant effects on

245   urinary IL-8. The "*Comamonas* × Magnesium" interaction effect was also significant, and it explained

246   73.00% of the variance in IL-8 concentration (Table S10). The strongest positive association between the

247   relative abundance of *Comamonas* and IL-8 concentration occurred in patients who reported a high

248   magnesium intake, and the weakest association occurred in patients who reported a low magnesium

249   intake (Figure 2B). This suggested that magnesium intake enhanced the relationship between *Comamonas*

250   and IL-8 concentration.

Discussion

252   During the last decade, evidence has accumulated to support a role for the microbiota, including the

253   bacterial communities in the gut and oral cavity, in T2DM patients (Navab-Moghadam et al., 2017;

254   Larsen et al., 2010; Zhang et al., 2018). Not only do T2DM patients have a different microbiota profile

255   from healthy subjects, but alterations in the microbiota are also responsible for immune responses, such as

256   the expression of proinflammatory cytokines (Xiao et al., 2017; Pushpanathan et al., 2016). Both human

257   and animal studies have shown that diet contributes to the composition of the gut microbiota (Egshatyan

258   et al., 2015; Wen & Duffy, 2017), and a gut microbiota signature that promotes intestinal inflammation

259   may exist, thereby promoting the development of T2DM (Wen & Duffy, 2017). Similar to previous

260   findings relating to the gut and oral microbiota, our previous research demonstrated that T2DM patients

261   have a different urinary microbiota composition from HCs, and the urinary microbiota composition was

262   correlated with urinary IL-8 concentration in T2DM patients (Liu et al., 2017; Ling et al., 2017). In

263   accordance with the "diet—gut microbiota—proinflammatory response" pathway described in previous

264   work, we investigated whether diet moderates the relationship between the urinary microbiota and IL-8

265   concentration.

266   In this study, the relative abundance of *Ruminococcus* was positively associated with urinary IL-8.

267   *Ruminococcus* spp. have rarely been reported in human urinary microbiota studies. A previous human

268   urinary microbiome study demonstrated that *Ruminococcus* was positively associated with preterm birth

269   (Ollberding et al., 2016), and it was detected in the urine of patients with chronic allograft dysfunction

270   (Wu et al., 2018). These indicate that urinary *Ruminococcus* might be associated with the occurrence and

271   development of some diseases. In our study, none of the subjects were diagnosed with UTI at recruitment

272   when their urine specimens were collected, but most samples having detectable *Ruminococcus* and IL-8.

273   This together with the positive correlation of *Ruminococcus* and urinary IL-8 level indicates that the

274   potential of members of *Ruminococcus* as a contributor of IL-8 and UTI in T2DM patients. Future studies

275   should investigate whether the UTI occurrence in the patients with detectable IL-8 is higher than those

276   with non-detectalbe IL-8.

277   Interestingly, water intake weakened the positive relationship between the relative abundance of

278   *Ruminococcus* and IL-8 concentration. Resolution of a UTI relies on the concentration of an antibiotic

279   within the urine, but highly concentrated urine reduced bactericidal activity against some pathogens

280   (Mikel & Marta, 2003). In a recent randomely controlled trial, increased water intake was effective on

281   controlling the number of cystitis episodes in women with reccurrent cystitis (Hooton et al., 2018).

282   Meanwhile, increased water intake can reduce the dose of antimicrobial-regimens (Hooton et al., 2018).

283   Surprisingly, increasing fiber intake enhanced the relationship between the relative abundance of

284   *Ruminococcus* and IL-8 concentration. In the gut, increased fiber intake improves the multiplication of

285   *Ruminococcus* (Abell et al., 2008), and dietary fiber was inversely associated with inflammatory markers,

286   including C-reactive protein (Wannamethee et al., 2009), interleukin-6 (Wannamethee et al., 2009; Qi et

287   al., 2006), TNF-α-R2 (Qi et al., 2006; Ma et al., 2008). Therefore, it seems that fiber intake contribute

288   inversely to the relationship of *Ruminococcus* with inflammatory cytokines from the gut to the urine..

289   Both vitamin B3 and vitamin E intake exerted an enhancing effect on the positive relationship

290   between *Ruminococcus* and IL-8 concentration. Since no previous studies reported that the roles of

291   vitamin B3 and vitamine E on either *Ruminoccocus* growth and IL-8 expression, future studies should be

292   focused on the role of the two vitamins in human urinary microbiome, then explore their moderation

293   effects on the correlation between urinary microbiome and inflammatory markers.

294   Cholesterol intake had significant enhancing effects on the abundance of *Comamonas* and urinary

295   IL-8. *Comamonas* is a bacterial genus in the phylum *Proteobacteria*. The current literatures supported

296   that *Comamonas* spp. was related to infections including phlegmon, peritonitis, endocarditis, meningitis,

297   septicemia, and UTI (Cooper et al., 2005; Biswas et al., 2014; Horowitz et al., 1990). Barua et al. found

298   that nephritic syndrome patients with higher serum cholesterol were more susceptible to UTI than those

299   with normal serum cholesterol, which might be because high cholesterol concentrations suppress

300   lymphocyte function (Barua et al., 2016). With regard to magnesium, its intake had an enhancing effect

301   on the relationship between the relative abundance of *Comamonas* and IL-8 concentration. However, it is

302   not recommended that T2DM patients should restrict magnesium intake, since a previous *in vitro* study

303   demonstrated that magnesium sulfate suppressed inflammatory response by human unbilical vein cells

304 (Rochelson et al., 2007; Weglicki et al., 1992), and a previous rodent model study demonstrated that

305 magenesium-deficiency elevated circulating levels of inflammatory cytokines (Weglicki et al., 1992).

306   There were several limitations in our present study.Firslty,the participants were only from Zhejiang

307 Province, China. It is known that different areas have different dietary patterns in China. For example, in

308 Chinese southern areas (incuding Zhejiang province), the body sizes of the local population are generally

309 small and short, and their diet is light and total intake is fewer than the northern areas. Thus, a multi-

310 centers study, including southern and northern areas in China, is needed to verify the results in the present

311 study. Secondly, we have failed to detect the co-effect of bacteria on the correaltion between urinary

312 microbiome and inflammatory reponse, since the 16S rDNA can only detect at the bacterial genus level

313 and it is impossible to culture bacterial genus to examine their co-effect. Thirdly, T2DM patients might

314 assess their dietary intake and exercise more accurately than the healthy subjects, because there were

315 monthly healthcare education programs for patients in the communities in Zhejiang province. During the

316 programs, the patients were instructed that dietary control and proper exercise could alleviate the progress

317 of diabetes. Therefore, the patients might underestimate their dietary intake and overestimate their

318 exercise. This might be one of the reasons why the patients in the present study had lower energy intake

319 and equall exercise time than the healthy subjects, while their values of BMI were not different. Also, the

320 response of assessment of dietary intake and and exercise might be different between the patients and

321 healthy subjects, which might be a bias for assessing urinary microbiome and IL-8 production.

Conclusions

323   To our knowledge, this study is the first study to demonstrate the moderating effects of food intake

324 on the relationship between the midstream urinary microbiota and urinary IL-8 concentration in humans.

325 It is suggested that modulating the dietary patterns of patients based on urinary microbiota dysbiosis

326 status and urinary IL-8 concentration might play a role in regulating inflammation response in urinary

327 tract system.

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466
467

Figure 1

Diet, Ruminococcus and IL-8

Figure 1

A

B

C

D

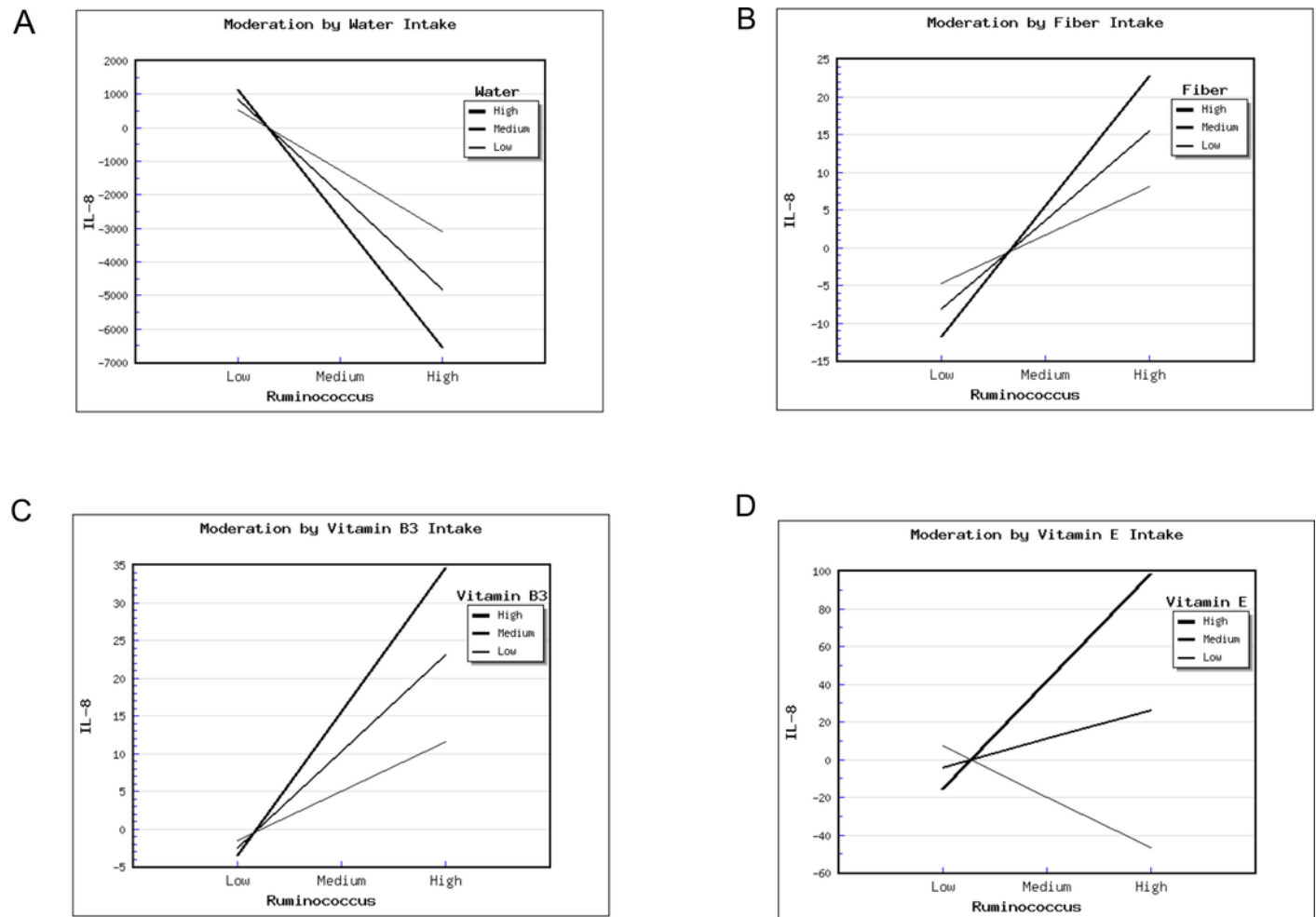



Figure 2

Diet, Comamomas and IL-8

Figure 2

A

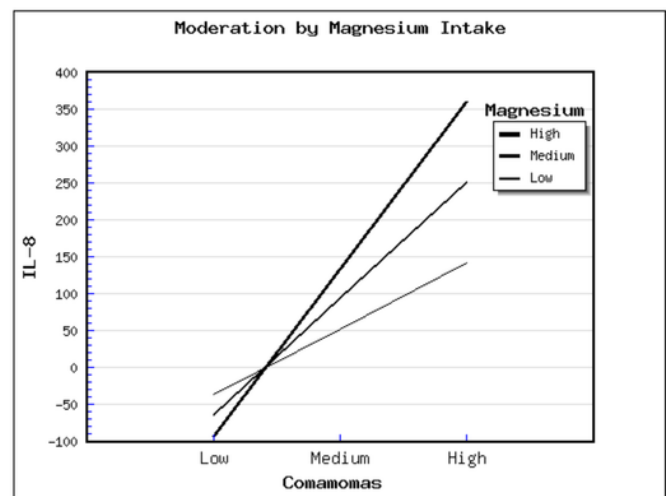
B

Characteristics of T2DM subjects

Table 1

1   Table 1 **Demographic and clinical characteristics of the study population**

Parameters	Value for corhort (n[a])[b] or statistic		p value[c]
	T2DM (n = 70)	HC (n = 70)	
Age (yr)			1.00
26-35	2 (2.86)	2 (2.86)	
36-45	6 (8.57)	6 (8.57)	
46-55	11 (15.71)	11 (15.71)	
56-65	17 (24.3)	17 (24.3)	
66-75	23 (32.9)	23 (32.9)	
$\geqslant$ 70	11 (15.7)	11 (15.7)	
Marital status [no. (%)]			1.00
Living with couple	64 (91.4)	64 (91.4)	
Living without couple	6 (8.6)	6 (8.6)	
Menstrual status [no. (%)]			1.00
Premenopause	11 (15.7)	11 (15.7)	
Postmenopause	54 (77.1)	54 (77.1)	
Hysterectomy	5 (7.1)	5 (7.1)	
BMI (kg/m^2)	23.87 ± 3.65	23.10 ± 4.49	0.27
Exercise[d]			0.15
Class 1	3(4.29)	4(5.71)	
Class 2	19(27.14)	17(24.29)	
Class 3	19(27.14)	30(42.86)	
Class 4	26(37.14)	14(2.00)	
Class 5	3(4.29)	5(7.14)	
Duration of T2DM (yr)	9.77 ± 7.49	NA	
FBG (mmol / L)	7.83 ± 2.35	5.22 ± 0.61	0.00
Urine pH	5.81 ± 0.62	5.93 ± 0.70	0.28
Urine protein POS [no. (%)]	14 (20.00)	3 (4.00)	0.01
Urine nitrite POS [no. (%)]	6 (8.57)	4 (5.71)	0.75
Urine leukocyte esterase POS [no. (%)]	13 (18.57)	18 (25.71)	0.42
Urine glucose POS [no. (%)]	14 (20.00)	0 (0.00)	0.00
UTI in the past 1 year [no. (%)]	27 (38.57)	7 (10.00)	0.00
Urine culture(E.coli)POS [no. (%)]	6 (8.57)	5 (7.14)	1.00

2   [a]n, no. of subjects.

3   [b]Mean ± SD or no. (%)

4   [c]Pearson's chi-square test or Fisher's exact test was used for categorical variables and *t* test was used to compare continuous

5   variables.

6   [d].taking exercise: Class 1:never; Class 2: several times per year; Class 3: several times per month, but every week; Class 4:

7   several times per week, but every day; Class 5: every day

8   Abbreviations: BMI: body mass index; E. coli:Escherichia coli; FBG: fasting blood glucose; HC: healthy control; NEG:

9   negative; POS: positive; T2DM: type 2 diabetes mellitus; UTI: urinary tract infections; yr: year;

Comparison of nutrient intake between T2DM and HCs

Table 2

1   Table 2 Comparison of nutrient intake between T2DM and HCs

Nutrients	T2DM	HCs	p-value
Water (mL / d)	2526.04 ± 903.79	2484.24 ± 829.94	0.79
Energy(kcal / d)	1256.55 ± 479.72	1469.07 ± 626.63	0.03
Protein(g / d)	60.73 ± 27.29	72.85 ± 46.09	0.06
Fat (g / d)	57.75 ± 30.05	59.32 ± 29.40	0.76
SFA (% TE / d)	11.65 ± 6.39	13.73 ± 11.29	0.18
MUFA (% TE / d)	18.67 ± 11.96	19.15 ± 11.32	0.81
PUFA (% TE / d)	16.71 ± 10.68	15.10 ± 8.68	0.33
Carbohydrate(g / d)	151.46 ± 58.59	184.38 ± 94.23	0.01
Fiber(g / d)	9.26 ± 4.54	12.50 ± 17.20	0.13
Cholesterol(mg / d)	472.36 ± 212.18	486.47 ± 179.68	0.67
Vit A(μgRE / d)	745.95 ± 315.96	1032.52 ± 1351.94	0.09
Retinol(μg / d)	265.07 ± 178.10	380.30 ± 632.97	0.15
Vit B1(mg / d)	0.70 ± 0.32	0.77 ± 0.32	0.18
Vit B2(mg / d)	0.88 ± 0.33	1.18 ± 0.93	0.01
Vit B3(mg / d)	13.92 ± 6.84	18.77 ± 15.09	0.02
Vit C(mg / d)	107.44 ± 40.45	127.10 ± 62.21	0.03
Vit E(mg / d)	13.83 ± 42.49	7.61 ± 20.65	0.27
Calcium(mg / d)	687.33 ± 323.02	831.39 ± 681.37	0.11
Phosphorus(mg / d)	936.87 ± 388.41	1090.99 ± 547.12	0.06
Potassium(mg / d)	1704.71 ± 678.50	2262.05 ± 1882.30	0.02
Sodium(mg / d)	2859.29 ± 1586.14	2788.47 ± 1585.96	0.79
Magnesium(mg / d)	267.73 ± 116.61	325.39 ± 233.91	0.07
Iron(mg / d)	21.47 ± 10.75	26.91 ± 24.19	0.09
Zinc(mg / d)	9.49 ± 3.84	11.47 ± 6.54	0.03
Selenium(μg / d)	38.42 ± 17.68	49.71 ± 29.51	0.01
Copper(mg / d)	1.78 ± 1.00	2.32 ± 2.47	0.10
Manganese(mg / d)	4.17 ± 1.80	8.95 ± 32.13	0.23

2   Abbreviations: TE: total energy intake; RE: retinol equivalent; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid;

3   PUFA: polyunsaturated fatty acid