Original Article

# Medium-chain triglycerides reduce diarrhea with improved immune status and gut microbiomics in tunnel workers in China

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**Background and Objectives:** Adverse environmental factors in tunnels increase the occurrence of respiratory and intestinal inflammatory disease, which is seriously harmful to worker health. It is reported that medium-chain triglycerides (MCT) can improve immune status and alter the gut microflora. This study investigates MCT effects on immune status and gut microbiota among tunnel workers. **Methods and Study Design:** Forty-five workers were randomly divided into an MCT group (n=30) and control group (n=15), where they ingested MCT-milk or a placebo milk for 12 weeks, respectively. The primary outcome measure was the incidence of respiratory infection and diarrhea. Secondary outcomes were changes in serum immune-related markers and changes in gut microbiota. **Results:** The incidence of diarrhea in MCT group was significantly decreased after 4 weeks ($p$<0.01), with no significant differences in the control group. MCT reduced the level of pro-inflammatory cytokines (TNF-α, CRP, and IL-6) and enhanced the anti-inflammatory cytokines (IL-10, C3, C4, IgA, IgG, and IgM), respectively ($p$<0.01). The Chao index was reduced ($p$<0.01) and microbiota composition changed significantly after 12 weeks of MCT intervention. MCT reduced the abundance of *Bacteroides*, *Roseburia*, *Ruminococcus_1*, *Lachnospira* and increased that of *Blautia* and *Fusicatenibacter* at the genus level ($p$<0.01). **Conclusions:** The consumption of MCT reduces diarrhea occurrence and improves serum immune profiles together with gut microbiomics in tunnel workers.

**Key Words: medium-chain triglycerides, tunnel environment, immunity, gut microbiota**

## INTRODUCTION

The tunnel working environment is different from the general outdoor construction environment, and its special natural environment factors and construction environment factors together constitute unique occupational disease risk factors for workers. The natural environmental factors include complex geological conditions, a confined working space, insufficient light, low oxygen content, humidity, and a large temperature difference, while construction environment factors are mainly reflected in mechanical noise, vibration, vehicular emissions, dust, and mental distress. These adverse environment factors can increase the incidence of respiratory and intestinal inflammatory disease, and seriously harm workers' health.[1-3] The gut microbiota of workers in the tunnel environment are altered by these adverse factors.[2] Medium-chain fatty acids (MCFA), such as caprylic acid (C8:0), capric acid (C10:0), and lauric acid (C12:0), are 6-12 carbon fatty acids, which occur naturally as medium-chain triglycerides (MCT) abundant in coconuts, palm kernels, cuphea seeds and human milk.[4] They are digested and absorbed in the stomach, catalyzed by lingual and gastric lipases, solubilized in the aqueous phase of the intestinal contents, transported to the liver via the portal vein, and rapidly metabolized with numerous physiological benefits, including metabolic regulation,[5] cognitive enhancement,[6] immune improvement,[7,8] and pathogen suppression.[9] MCFA can alter gut microbiota and affect host inflammation, obesity, inflammatory bowel disease, type 2 diabetes, and cardiovascular disease,[10] However, human studies are limited. In animal studies, we have found that MCT improves immunity and has anti-inflammatory effects,[8] and others have reported that parenteral nutrition with MCT-containing formulations improves the inflammatory factor profiles in preterm infants.[11] Immunoinflammatory status after long-term consumption of MCT is unclear. The present study investigates the effects of MCT on such status

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and the gut microbiota in long-term tunnel workers at risk of respiratory and gut inflammatory disease.

## METHODS

### *Study design and population*

This study was approved by the Chinese People's Liberation Army General Hospital Ethics Committee and registered at the Chinese Clinical Trial Registry Website (ChiCTR1900026595). Written informed consent was obtained from all participants before enrollment. Potential recruits were workers in a tunnel construction unit, all of whom had been involved in tunnel construction for more than 1 year.

Inclusion criteria were: 1) male aged 18-35; 2) participant in tunnel work for more than 1 year; 3) an average daily working time of more than 8 hours. Exclusion criteria were: 1) an infection-related illness or have taken antibiotics, probiotics, immune-boosting foods and medicines within 2 weeks prior to study participation; 2) body weight change of more than 10% in the past year, or chronic dieting or overeating; 3) medical conditions, including hypertension, cerebrovascular disorders, heart or pulmonary diseases, diabetes, endocrine, gastrointestinal, or psychiatric disease; 4) allergy to dairy products and lactose intolerance; 5) unable or unlikely to complete the intervention or work in the tunnel environment for the next 12 weeks.

Workers were randomly assigned to either a control or MCT group with a ratio of 1:2. Interventions were either milk (227 mL per serving) or MCT-milk (227 mL per serving) which contained 7g MCT (58% of caprylic acid and 42% of capric acid). Both interventions were provided by the Beijing Sanyuan Food Co., Ltd. The two products had the same outer packaging, but different production batch numbers in order to both blind and reliably assign them. All participants consumed a 227 mL serving twice per day, with breakfast and dinner, for 12 weeks. Consumption was supervised. Workers, questionnaire administrators and data analysts were blinded. At study conclusion, the investigators and intervention providers jointly conducted the unblinding. All participants lived in the same building, ate in the same cafeteria, and had similar working hours, workplaces, and intensity of daily work.

Assessments were undertaken on 4 occasions, at 0wk (baseline), 4wk, 8wk and 12wk. At each visit, dietary intake, occurrence of respiratory infection and diarrhea in the previous 4 weeks were documented by questionnaire. Fasting blood sampling and morning stool collection were also undertaken at the 0wk and 12wk visits.

### *Biochemical measurements*

Blood samples were collected in vacutainer tubes containing serum separator and immediately centrifuged at 2,000 g for 10 min at 4°C. Serum were frozen at -20°C, and stored in a -80°C freezer the next day until analysis. Immunoglobulins (IgA, IgM, IgE, and IgG), complement (C3 and C4), interleukins (IL-1, IL-2, IL-6, IL-8, and IL-10), tumor necrosis factor alpha (TNF-α), and C-reactive protein (CRP) were analyzed in accordance with the manufacturer's instructions (Shanghai Enzyme-linked Biotechnology Co., Ltd, Shanghai, China), and detected with a microplate reader (Rayto RT-6100, Rayto Life Science Co., Ltd. Shenzhen, China). Assays were performed in duplicate.

### *DNA extraction and PCR amplification*

DNA was extracted from fecal samples with the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany). We conducted tag-pyrosequencing analysis of the V3–V4 region of 16S rRNA gene to identify intestinal bacteria. We amplified this region using the broadly conserved primers, 338F (5'-GGACTACHVGGGTWTCTAAT-3') and 806R (5'- GGACTACHVGGGTWTCTAAT-3'), containing the A and B sequencing adaptors. Different barcode sequences were used to tag these primers to analyze multiple samples. Each sample reaction mixture (20 μl) contained 0.5 μl of 5 U/μl Easy Taq DNA polymerase, 2 μl of 10 × Easy Taq buffer, 2 μl of 0.25 mmol/L dNTPs, 0.2 μmol/L of each primer, 10 ng of template DNA, and deionized ultrapure water (to 20 μl). An Applied Biosystems GeneAmp PCR System 9700 was used to amplify the DNA samples as follows: initial denaturation at 94°C (3 min) followed by 27 cycles at 95°C (30 s), 55°C (30 s), and 72°C (45 s), and final extension at 72°C for 10 min. We used a 2% (w/v) Tris-Boric acid-EDTA (TBE) agarose gel to assess the quality of the amplicons.

### *Illumina MiSeq sequencing*

The amplicons were purified using a MiniElute PCR purification kit (Axygen) and quantified using the Applied Biosystems GeneAmp PCR System 9700. The PCR products were pooled at equal concentrations and pyrosequencing, and performed using an Illumina HiSeq 2500 platform (Novo gene, Beijing, China).

### *Assessment of outcomes*

The primary outcome was the incidence of respiratory infection or diarrhea, as assessed at baseline, 4wk, 8wk, and 12wk by questionnaire. Secondary outcomes were the changes in immune-related markers in serum and changes in gut microbes, assessed at baseline and 12wk.

### *Bioinformatics and statistical analysis*

Continuous variables are reported as mean ± standard deviation and Student t test used to test differences between the groups; while discrete variables are reported as frequency (%) and McNemar's test or Fisher's exact test used to analyze differences between the groups.

The processing method for the biological information of the fecal flora was as follows: All sequences acquired using the Illumina HiSeq 2500 were saved in the raw fastq files. The initial processing of the raw dataset included screening to remove short and low-quality reads; and only high-quality sequences without primer sequences were retained. The 16S rRNA gene extracted from fecal samples was amplified following the standard procedures of the Earth Micro biome Project using a set of updated universal primers 515F/806R specifically targeting the hyper variable V4 region. The V4 region was sequenced by the Illumina MiSeq (San Diego, CA, USA) and then processed through the workflow package Quantitative Insights into Microbial Ecology (QIIME ,version 1.8.0).[12] The operational taxonomic units (OTUs) were

picked using a 97% similarity threshold as the criterion to identify the OTUs by clustering analysis in QIIME.[13] Before downstream analysis, taxonomic classification and quantification of OTUs were performed against the Greengenes version 13.8 16S rRNA database.[14,15] At least two OTU sequences (singletons) were discarded to the final OTUs table and then sequences were aligned using a PyNAST aligner.[12] T-test was used to analyze if there were any significant differences in the relative proportion of microbial community compositions between treatments. For alpha diversity indexes (Shannon), Wilcoxon's rank-sum test was applied to assess species diversity and evenness. Principal coordinate analysis (PCoA) based on the weighted Unifrac metric was conducted to determine the beta-diversity of different bacterial communities in each treatment using QIIME and visualized by SigmaPlot version 10.0. The beta-diversity of microbial communities was assessed for the significant differences by Adonis test based on R Studio software (version 7.2). For statistical analysis of the distribution of common and special OTUs, Venn assessment was performed among four groups.16 In addition, we calculated the mean, standard error, and standard deviation of resultant data using Microsoft Excel version 2010 (MS, USA). Spearman correlation analysis was used to evaluate the associations of the differential microbial species with the measured inflammatory factors.

All statistical analysis was performed with SPSS 16.0 for windows (IBM, USA) unless otherwise indicated. A two-sided $p$ value <0.05 was considered statistically significant, and a two-sided $p$ value <0.01 was considered extremely significant.

## RESULTS

### *Overall sample description*

Forty-five workers were included and completed the study. They were divided randomly into a control group (n=15) and an MCT group (n=30) with a ratio of 1:2; all completed the entire research protocol, including venipuncture. Four people in the control group and 2 in the MCT group provided no stool sample at the end of the study, so these individuals were not included in the gut microbiota analysis.

### *Clinical characteristics*

There were no significant differences between the two groups in age, anthropometric, personal behavioral (smoking, drinking), or metabolic indicators at the beginning of the study (Table 1).

### *Primary outcome*

The incidence of diarrhea in the MCT group decreased by 4wk, 8wk, and 12wk compared to baseline ($p$<0.01), with no recognizable changes in other indicators ($p$>0.05). There were no significant differences between the two groups at any of the 4 visits (Table 2).

### *Effects of MCT on serum inflammatory markers*

After 12 weeks of MCT, the concentrations of IL-10, C3, C4, IgA, IgM, and IgG significantly increased compared with baseline ($p$<0.01), and the concentrations of IL-1, IL-6, TNF-α, and CRP were significantly reduced compared with baseline ($p$<0.01), respectively. These trends were not observed before or after placebo. At the end of the study, the concentrations of IL-10 ($p$<0.01) and C3 ($p$<0.05) in the MCT group were significantly higher than in the control group, while the concentration of IL-1, IL-6 and CRP were significantly lower than in the control group ($p$<0.05). The concentration of TNF-α in the MCT group was significantly higher than in control at the beginning of the study; and, curiously, decreased further during the study in the control group ($p$<0.01) (Figure 1).

### *Effects of MCT on fecal microbial composition*

We collected 22 fecal samples from 11 workers in the control group and 56 fecal samples from 28 in the MCT group before and after the intervention, respectively. From these 78 samples, a total of 4,313,332 sequences (mean length: 416 bp) were mapped to 741 OTUs. For alpha diversity analysis, the number of observed species (Figure 2A) and the Chao index in the MCT group were significantly lower than in control group at the start and end of the study ($p$<0.01). The Chao index of MCT group

**Table 1.** Behavioral, anthropometric, and metabolic and haematological characteristics by intervention

| Characteristic | Control (N=15) | MCT (N=30) | $p$ value† |
|---|---|---|---|
| Age (years) | 23.1±3.86 | 24.8±3.92 | 0.160 |
| Body height (cm) | 175±6.03 | 174±5.72 | 0.462 |
| Body weight (kg) | 69.0±7.71 | 71.7±12.01 | 0.433 |
| BMI (kg/m$^2$) | 22.5±2.68 | 23.7±3.26 | 0.242 |
| Smoking (n/%) | 8 (53.3) | 17 (60.7) | 0.832‡ |
| Drinking (n/%) | 0 (0) | 0 (0) | NA |
| TG (mmol/L) | 0.78±0.40 | 1.00±0.45 | 0.132 |
| TC (mmol/L) | 3.72±0.82 | 3.95±0.81 | 0.368 |
| TP (g/L) | 72.0±3.00 | 73.0±2.79 | 0.259 |
| ALB (g/L) | 49.7±2.42 | 50.1±2.17 | 0.546 |
| GLOB (U/L) | 22.3±1.63 | 22.9±2.70 | 0.435 |
| WBC (10$^9$/L) | 6.2±1.10 | 5.8±0.87 | 0.246 |
| RBC (10$^{12}$/L) | 4.7±0.27 | 4.7±0.27 | 0.375 |
| Hb (g/L) | 160±12.5 | 165±8.90 | 0.149 |
| PLT (10$^9$/L) | 172±16.8 | 178±27.2 | 0.395 |

ALB: albumin; BMI: body mass index; GLOB: serum globulin; Hb: hemoglobin; MCT: medium-chain triglycerides; NA: not applicable; PLT: Platelet; RBC: red blood cell; TC: total cholesterol; TG: total triglycerides; TP: total protein; WBC: white blood cell.
†T-test was used unless otherwise indicated.
‡Chi-square test.

**Table 2.** Respiratory infection and diarrheal incidence by visit and MCT intervention among tunnel workers

| | Control (N=15) | | MCT (N=30) | | *p* value (Between-groups) |
|---|---|---|---|---|---|
| | n (%) | *p* value (Within-groups) | n (%) | *p* value (Within-groups) | |
| Respiratory infections | | | | | |
| Baseline | 2 (13.3) | Reference | 4 (13.3) | Reference | 1 |
| 4wk | 2 (13.3) | 1 | 1 (3.3) | 0.180 | 0.254 |
| 8wk | 0 (0) | NA | 0 (0) | NA | NA |
| 12wk | 1 (6.7) | 0.317 | 1 (3.3) | 0.180 | 1 |
| Diarrhea | | | | | |
| Baseline | 4 (26.7) | Reference | 12 (40.0) | Reference | 0.514 |
| 4wk | 3 (20.0) | 0.655 | 2 (6.7) | 0.004 | 0.314 |
| 8wk | 2 (13.3) | 0.157 | 2 (6.7) | 0.002 | 0.591 |
| 12wk | 2 (13.3) | 0.157 | 1 (3.3) | 0.001 | 0.254 |

NA: no available incidence to analyze.
McNemar's test was used for within-groups comparison, and Fisher's exact test was used for between-groups comparison.

significantly decreased after 12 weeks intervention ($p$<0.01) (Figure 2B). However, the Shannon index exhibited no significant difference among the 4 groups (Figure 2C). Interestingly, we obtained 497 bacterial OTUs in control-str group, 552 in control-end group, 354 in MCT-str group, and 445 OTUs in MCT-end group, among which 33, 81, 11, and 68 OTUs were unique in each group. (Figure 2D). From the principal coordinates analysis (PCoA), the compositions of gut microbiota in the MCT-str and MCT-end group were well separated on the PC1 axis, emphasizing the large shift in microbiota composition after MCT intervention for 12 weeks, which explained 42.66% of the total variance observed in PC1. However, the gut microbiota in none of the four groups separated from each other on PC2 axis, which accounted for 12.58% of the total variance (Figure 2E).

Compositions are shown for the top 10 phyla (Figure 3A), class (Figure 3B), order (Figure 3C), family (Figure 3D) and genus (Figure 3E). The particular genera that changed after MCT intervention were: the abundance of Blautia (Figure 3F) and *Fusicatenibacter* (Figure 3K) which significantly increased (p<0.05), while the abundance of *Roseburia* (Figure 3G), *Ruminococcus_1* (Figure 3H), *Bacteroides* (Figure 3I), and *Lachnospira* (Figure 3J) significantly decreased ($p$<0.01).

***Gut microbiome variation induced by MCT and associated with serum immunoinflammatory markers***

After identifying the gut microbiome signature, we explored whether or not microbiomic perturbation was associated with immunoinflammatory markers as judged by Pearson correlation analysis between serum parameters and microbial species at genus level (Figure 4). TNF-α had a positive association ($p$<0.01) with *Ruminococcus_1*, *Paraprevotella*, *Megasphaera*, and *Tyzzerella_3*, while there was an opposite association ($p$<0.01) with *Anaerostipes*; in addition, it positive correlated positively ($p$<0.05) with *Coprococcus_2* and *Lachnospira*. CRP was positively associated ($p$<0.01) with *Lachnospira*, *Tyzzerella_3 Megasphaera*, and *Paraprevotella*, while oppositely associated ($p$<0.01) with *Collinsella*, *Anaerostipes*, *Dorea*, and *Fusicatenibacter*; in addition, it was positively correlated ($p$<0.05) with *Collinsella* and oppositely ($p$<0.05) with *Blautia*. IgA was negatively associated ($p$<0.01) with *Paraprevotella*, *Megasphaera*, *Tyzzerella_3*, and *Lachnospira,* and positively ($p$<0.05) with *Collinsella*, *Anaerostipes*, and *Dorea*. IgE was negatively associated ($p$<0.05) with *Anaerostipes*, *Dorea*, and *Fusicatenibacter*. The IgG positively with *Collinsella* ($p$<0.01) and *Dorea* ($p$<0.05), with opposite correlations ($p$<0.05) with *Tyzzerella_3* and *Lachnospira*. IgM was positively associated ($p$<0.05) with F*usicatenibacter*, and oppositely ($p$<0.05) with *Megasphaera.* IL-2 was oppositely associated ($p$<0.05) with *Anaerostipes* and *Dorea.* IL-6 was positively associated ($p$<0.05) with *Megasphaera*. The IL-10 was oppositely associated ($p$<0.01) with *Paraprevotella*, *Megasphaera*, *Tyzzerella_3*, and *Lachnospira*, and oppositely correlated with *Coprococcus_2*. However, it was positively associated ($p$<0.01) with *Collinsella*, *Anaerostipes*, and *Dorea*, and positively ($p$<0.05) with *Blautia*. C3 was negatively associated ($p$<0.01) with *Lachnospira*, *Tyzzerella_3*, P*araprevotella*, *Roseburia*, *Coprococcus_2*, and *Lachnospiraceae_UCG-004*, and oppositely correlated ($p$<0.05) with *Megasphaera* and *Bacteroides*; it was positively correlated ($p$<0.01) with *Collinsella*. C4 had a negative association ($p$<0.01) with *Tyzzerella_3*, and an opposite correlation ($p$<0.05) with *Lachnospira*.

## DISCUSSION

***Occupational illness prevention with MCT***

Although dietary intake of MCT or MCFA is known to improve immunity in animals or cells, effects on human immune status are less well established.[17] Our study to demonstrate that medium-term (12 weeks) dietary MCT supplementation can reduce the incidence of diarrhea and improve immune status in the health vulnerable conditions of tunnel workers. This finding must be treated circumspectly, however, since the study sample size was relatively small and the intervention period of medium duration. The failure to recognize an association of MCT consumption with respiratory illness may be a type 2 error attributable to sample size.

***MCT consumption and immunoinflammatory markers with occupational vulnerability***

Fu et al demonstrated that *Cinnamomum camphora* seed kernel oil with 90% MCFA (51.49% of capric acid and 40.08% of lauric acid) decreased the blood TNF-α and IL-6 level in obese rats.[18] In another study, Du et al

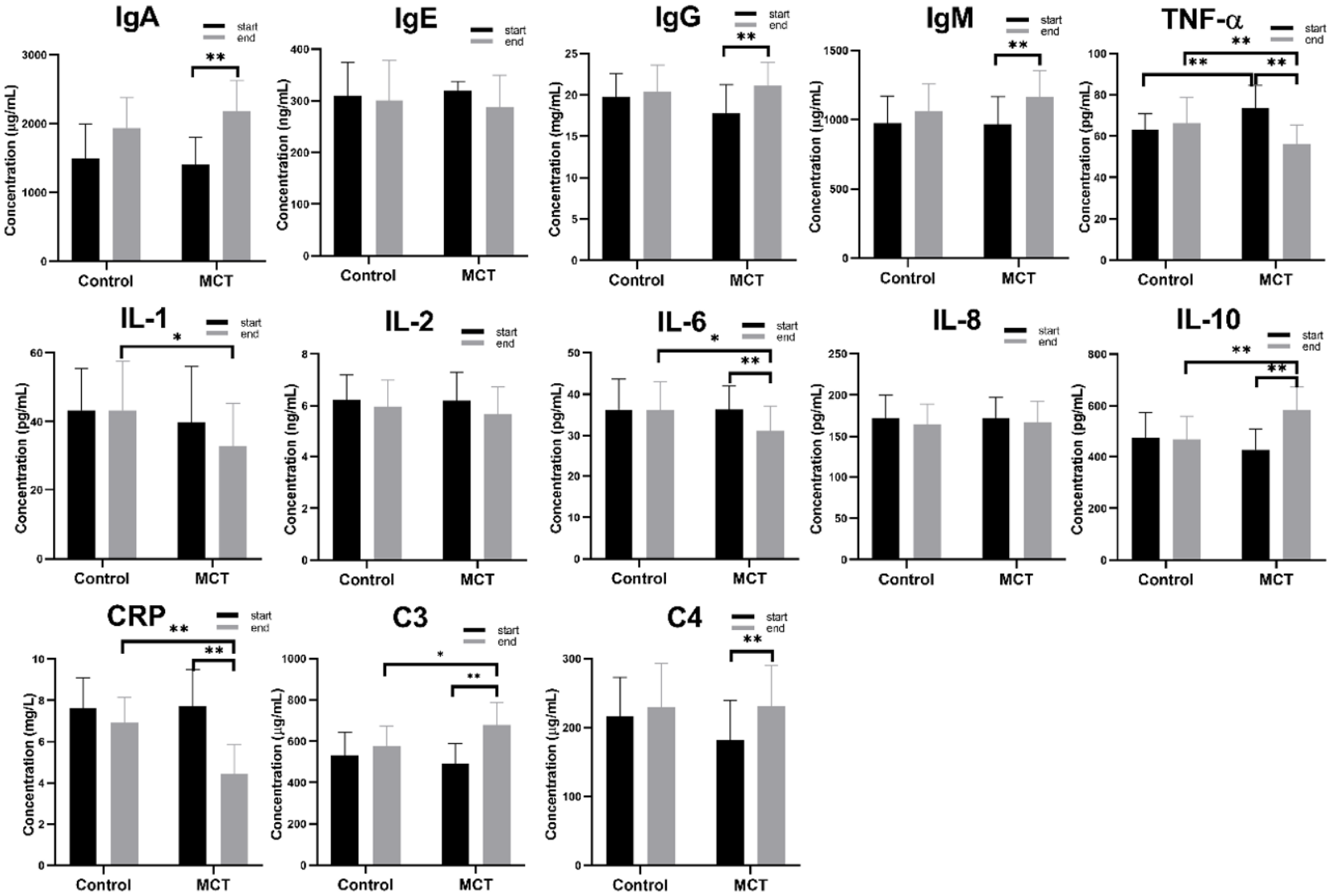


**Figure 1.** Effect of MCT on serum immunoinflammatory indicators of tunnel workers. The figure shows the changes of concentration of IgA, IgE, IgG, IgM, TNF-α, IL-1, IL-2, IL-6, IL-8, IL-10, CRP, C3, and C4 after 12 weeks intervention, respectively. Black column and grey column indicate the start and end of the study, respectively. Paired samples t-test was used for intra-group comparison, and independent two-sample t-test was used for comparison between two groups. $^{*}p<0.05$, $^{**}p<0.01$.

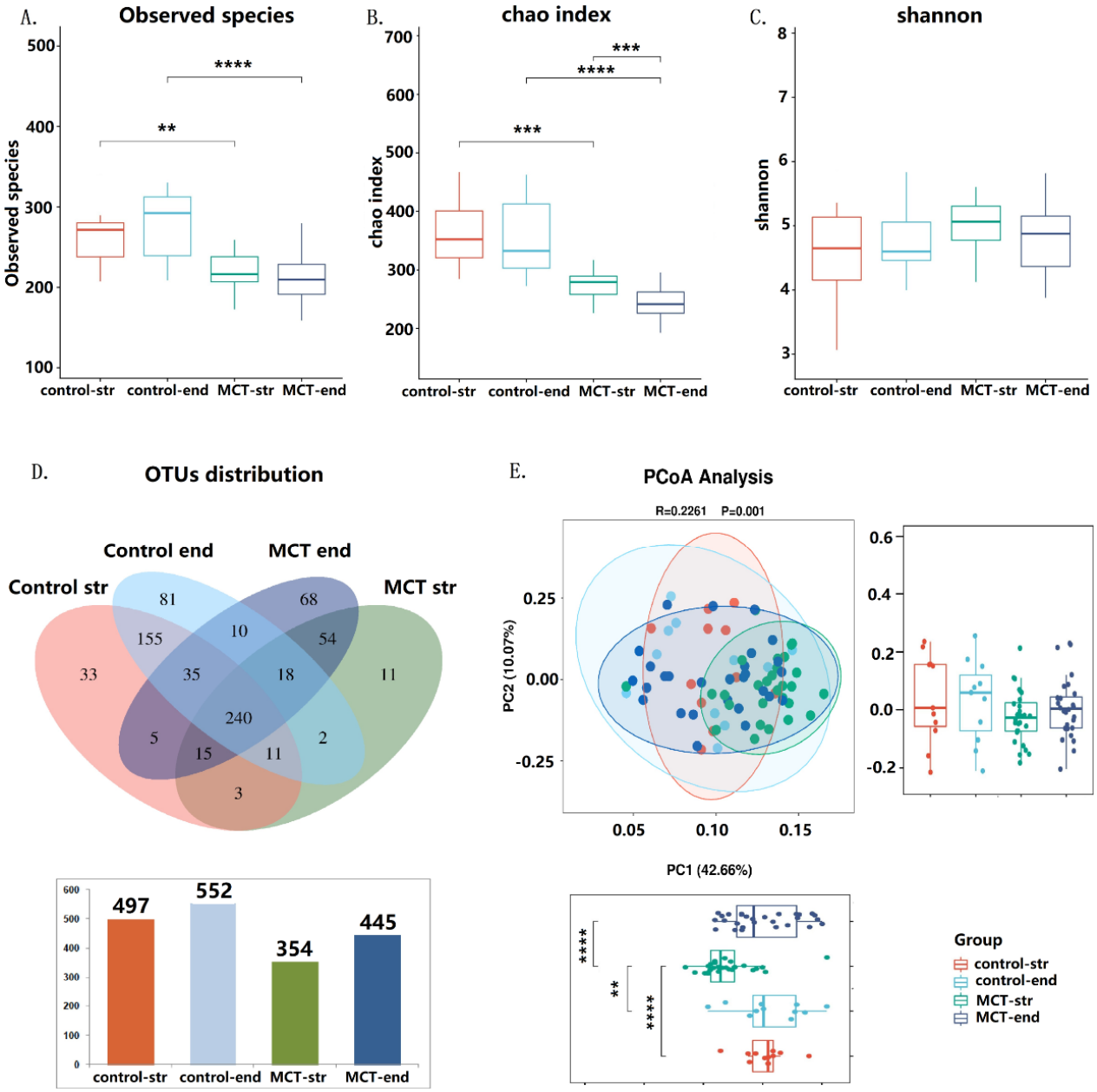


**Figure 2.** Supplementation altered the structure of intestinal microbiota. α-diversity: (A) observed species, (B) Chao index, (C) Shannon index and (D) OTU (Operational Taxonomic Unit) Venn diagram between treatments; β-diversity: (E) Principal coordinates analysis (PCoA) based on unweighted UniFrac distances is shown along the first two principal coordinate (PC) axis with Adonis *p* value. Percentages are the percent variation explained by each PC axis. ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$).

found similarly that being fed for 6 weeks with MCT (47.9% of caprylic acid and 52.1% of capric acid) significantly reduced endotoxin, TNF-α, and monocyte chemoattractant protein-1 (MCP-1) in plasma, with lower IL-6, TNF-α and higher IL-10 in both liver and white adipose tissue in high fat diet-induced obese rats.[19] Papada et al found that being pre-fed for 12 days with a MCT-enriched diet significantly decreased IL-6, IL-8 and intercellular adhesion molecule-1 (ICAM-1) of colon in the rat model of trinitrobenzene sulphonic acid colitis, which mimics human inflammatory bowel disease.[20] Similarly, in mouse experiments, Sadeghi et al reported that being pre-fed for 5 weeks with a MCT-enriched diet (6.8% of capric acid and 56.5% of lauric acid) significantly decreased peak plasma TNF-α, IL-1β and IL-6 and increased the peak plasma IL-10 concentration after intraperitoneal nonlethal Escherichia coli LPS in mice.[21] Geng et al found that MCT (67% of caprylic acid, 23% capric acid, and 10% other MCT) suppressed upregulation of IL-6 and downregulation of IL-10 in high fat diet-induced obese mice.[22] Kono et al fed MCT through a feeding tube and showed an increase in expression of IL-6, followed by secretion of immunoglobulin A (IgA) after the injection of bacterial LPS.[23] In the same study, the LPS-induced expression of proinflammatory cytokines and chemokines (such as TNF-α, IL-18, and MCP-1) were lowered by MCT and the expression of the immune modulating and anti-inflammatory cytokine IL-10 in ileum and Peyer's patches was greater in the MCT group. Wu et al. found that MCFA (lauric acid) upregulated serum immunoglobulins (IgA, IgM, and IgY), and downregulated inflammatory cytokines (IL-1β, IL-6, TNF-α, IL-4, and IL-10) in broilers.[24] Conversely, Bai et al reported that dietary supplementation with coconut oil decreased plas-

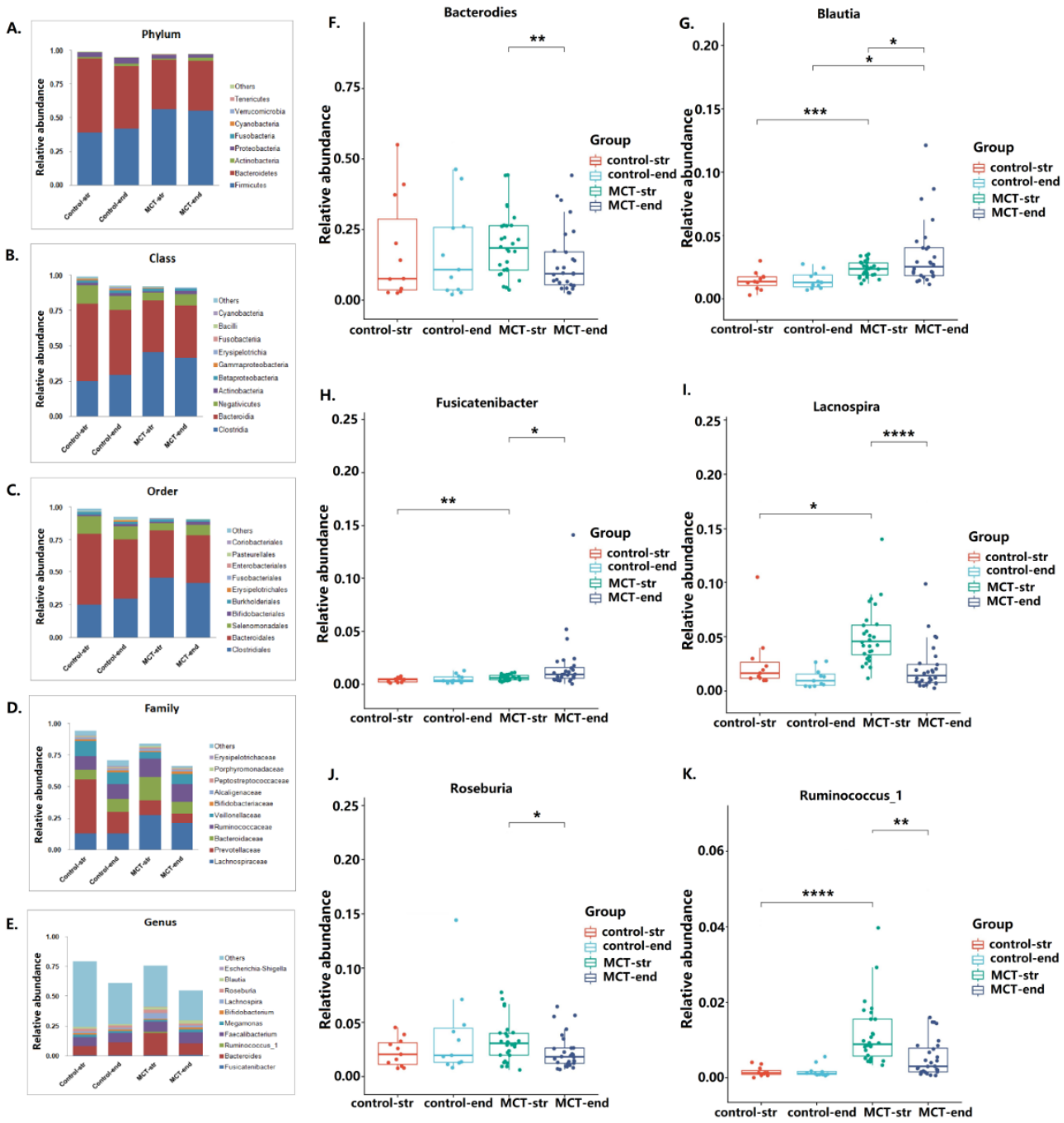


**Figure 3.** Comparison of bacterial community compositions based on bacterial OTUs (Operational Taxonomic Unit) between different treatments. The relative abundance of top 10 phylum (A), class (B), order (C), family (D), genus (E) and the significantly different species at genus level (F-K). ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$, the $p$ value was adjusted using the Bonferroni method).

ma IgG and IgA in piglets.[25] Any possible relationship between MCT or MCFA and human immunity has been uncertain. Pietraszek et al investigated the acute effects of dietary fat on immune biomarkers and found that plasma IL-6 progressively increased whereas the high-sensitivity acute-phase reactant C-reactive protein (hs-CRP) was unchanged for 240 min after ingestion of an MCT-enriched meal containing 40g coconut oil in 34 people.[26] Bohl et al. found no changes in circulating inflammatory markers after MCT consumption for 12 weeks in people abdominally obese.[27]

Effects of MCT or MCFA on the immune system have been found inconsistent, with differences in setting, dose, and study design (such as time frame and indicators). However, caprylic acid apparently exhibits anti-inflammatory effects, while capric acid exhibits pro-inflammatory effects. Sam et al have reported that capric acid increases the IL-1β, IL-6, and TNF-α production while IL-10 production decreases in human peripheral blood mononuclear cells co-stimulated with Candida albicans.[28] Similarly, Tanaka et al observed that capric acid enhanced IL-8 production in human Caco-2 cells.[29] Hoshimoto et al reported that caprylic acid suppressed IL-8 secretion in Caco-2 cells after 24 h preincubation by inhibition of the IL-8 promoter.[30] Zhang et al found caprylic acid to inhibit inflammatory cytokine expression of TNF-α and MCP-1, and increase plasma IL-10 in ApoE-deficient mice.8 Perhaps caprylic acid, but not capric acid, suppresses inflammation, but the mechanisms possibly involved remain unclear. In our study, we observed that

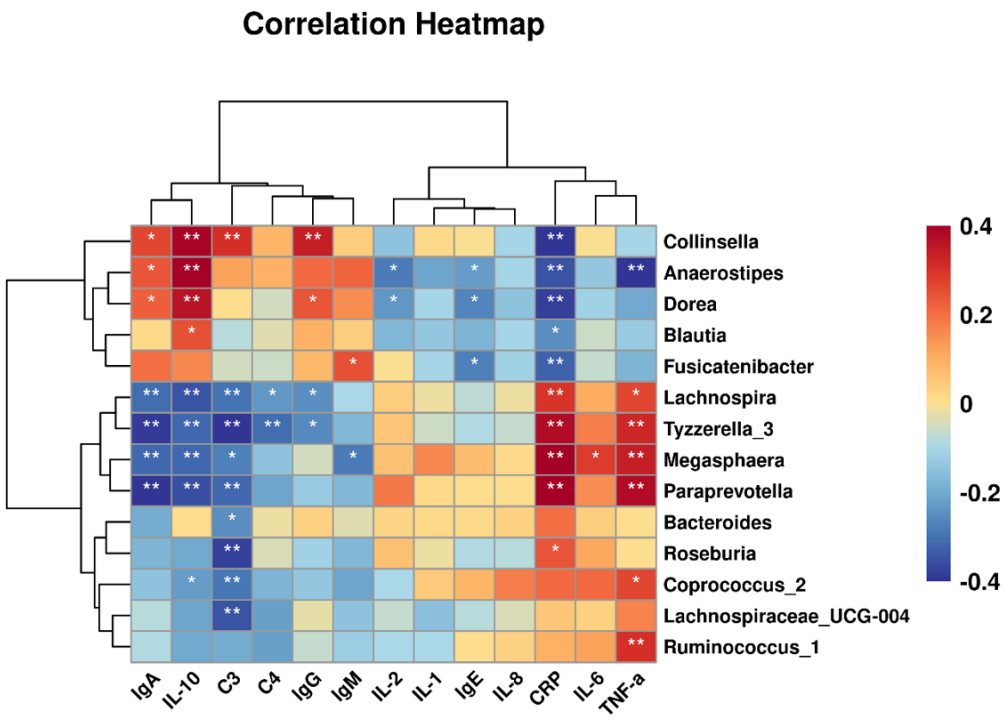


**Figure 4.** The Pearson correlation analysis was applied to analysis the associations of the differential microbial species at the genus level with the measured inflammatory factor. The heat map of correlation coefficient, the red represents positive correlation, and the blue represents negative correlation, respectively ($^{*}p<0.05$, $^{**}p<0.01$).

serum anti-inflammatory factors such as IL-10, C3 and C4 increased with MCT intervention, and pro-inflammatory factors such as TNF-α, CRP, IL-2, and IL-6 decreased, consistent with available animal and human reports.[11,19-22,24,31] The high content of caprylic acid in the MCT used in our study may have contributed an anti-inflammatory effect.

***MCT consumption and gut microbiomics***

The linkages between dietary quality, microbiomic diversity, and human health are increasingly well documented and involve short chain fatty acids (SCFAs).[32,33] Both dietary pattern and nutrients can influence the immune system directly, but may also modulate it indirectly by regulating the gut microbiota.[34,35] A healthy intestine is dependent on a balance between pro- and anti-inflammatory signals to be able to provide tolerance to beneficial bacteria and meanwhile fight against intestinal pathogens.[36] When the gut microbiota is disturbed, microbial-associated molecular patterns (such as LPS and bacterial lipoprotein) can activate immune cells and toll-like receptors (TLR) to trigger the release of pro-inflammatory cytokines.[37] There is growing evidence that MCT or MCFA can modulate the gut microbiota in animals and affect their health, some studies have reported that MCT or MCFA has a natural inhibitory effect on intestinal pathogens.[38-41] Lopez-Salazar et al report that consumption of coconut oil leads to lower bacterial diversity compare to olive oil or soybean oil.[42] Similarly, in our study the alpha-diversity of intestinal flora decreased after MCT intervention, which was reflected in a decrease of the Chao index.

To further explore the changes of gut microbiota, we conducted an in-depth analysis at the genus level and found *Blautia*, *Fusicatenibacter*, *Roseburia*, *Ruminococcus_1*, *Lachnospira*, and *Bacteroides* were significantly changed after MCT intervention.

*Blautia*, as a dominant genus in the intestinal microbiota, has a significant correlation with host physiological dysfunctions, such as obesity, diabetes, cancer, and various inflammatory diseases.[43] *Blautia* plays the role of an antibacterial agent and prevents inflammation by upregulating intestinal regulatory T cells and producing SCFAs.[44] However, higher abundance of *Blautia* has been found to be positively associated with the irritable bowel syndrome.[45] Yue et al demonstrated that the alleviating effects of medium-, long-, and medium-chain (MLM) structured lipids supplementation on atherosclerosis (AS) in high-fat diet-fed ApoE-/- mice were closely related to increase of *Blautia* abundance in the gut and decreased serum TNF-α.[46] Similarly, *Blautia* was found to be increased after the MCT intervention in our study, in turn positively correlated with IL-10 and negatively correlated with TNF-α. We speculate that MCT may exert anti-inflammatory effects by increasing *Blautia*, due to upregulation of intestinal regulatory T cells and SCFA production.

*Fusicatenibacter*, is a SCFAs-producing bacteria, closely related to host health, including ulcerative colitis, allergic rhinitis, functional constipation and Parkinson's disease;[47-49] it suppresses intestinal inflammation and is positively associated with SCFA production and IL-10 level.[50] It has been found that an increase of *Fusicatenibacter* is associated with improvement in diarrheal symptoms.[51] Again, *Fusicatenibacter* helps regulate colonic motility, perhaps attributable to modulation by fecal butyrate and serum IL-8.[48] Consistent with this, we found that MCT intervention increased the abundance of *Fusicatenibacter* and was negatively correlated with serum CRP, so improving the immune status of tunnel workers.

Likewise, Roseburia, as a commensal bacterium producing SCFAs, especially butyrate, affects colonic motility, chronic kidney disease progression, immunocompetence, and is anti-inflammatory.[52-55] Jiang et al identified a negative correlation between *Roseburia* and CRP and renal function, which suggests that the depletion of butyrate producing bacteria may contribute to CKD-associated inflammation and CKD progression.[54] Bajaj et al found that hepatic encephalopathic patients had less *Roseburia*, which was associated with decreased inflammation of the mucosal microbiome. Similarly, we found that the abundance of *Roseburia* to be reduced after MCT intervention. In addition, the change of *Roseburia* was positively correlated with the change in serum CRP, but negatively correlated with that in serum C3.

The genus *Lachnospiras*, as well as *Roseburia*, both within the phylum *Firmicutes*, are known to be producers of SCFAs, and involved with host immunity.[56] Luo et al reported that *Lachnospira* was remarkably decreased in newly diagnosed tuberculosis (NTB) and in recurrent tuberculosis (RTB) compared with healthy people, and positively related to CD4+ counts in NTB, but was negatively related to CD4+ counts in RTB.[57] Zhu et al found the relative abundance of *Lachnospira* to be positively correlated with diarrhea as well as IgM in piglets.[58] Xu et al demonstrated that L-theanine (found in tea) increased the proportion of *Lachnospira* while increasing total SCFAs content of feces, along with IgA, IgE, and IgG in the ileum of Sprague-Dawley rats.[59] However, in our study, we found that the relative abundance of *Lachnospira* was reduced by MCT intervention, positively correlated with pro-inflammatory factors (CRP and TNF-α), and negatively correlated with anti-inflammatory factors (IgA, IgG, C3, C4, and IL-10). This suggests that a reduction in the relative abundance of *Lachnospira* improves the immune status of tunnel workers.

*Ruminococcus* is a genus of bacteria in the class *Clostridia*. They are anaerobic, gram-positive gut microbes. They play an important role in digesting resistant starch, but are also associated with intestinal diseases (e.g., IBS, IBD, CD), immune diseases (e.g., allergies, eczema, asthma) and neurological diseases (e.g. autism, depression).[60-67] Geng et al found that fed weaned piglets with the *Lactobacillus rhamnosus GG ATCC53103* and *Lactobacillus plantarum JL01* reduced the relative abundance of *Ruminococcus_1* and *Ruminococcaceae_UCG-005* in cecum, while increasing expression of IL-10 and TGF-β1 mRNAs.[62] O'Sullivan et al. showed that formula-fed infants had more *Ruminococcus* and higher TNF-α, IFN-γ, IL-1β, IL-4, and other cytokines compared with breast-fed infants.[68] But reports on the effect of MCT or MCFA on the *Ruminococcus* genus are inconsistent. Yue et al. reported that MLM structured lipids supplementation could reduce the abundance of *Ruminococcus* in high-fat diet-fed ApoE-/- mice,[46] while Zhang et al reported glycerol monocaprylate (as a glycerol derivative of MCFA) increased the abundance of *Ruminococcus* in C57BL/6 mice.[41] In our study, *Ruminococcus_1* was decreased after MCT intervention, and that this was positively related to TNF-α. This suggests that MCT might improve the immune status of tunnel workers by downregulating Ruminococcus_1 and TNF-α.

*Bacteroides* is a genus of gram-negative anaerobic bacteria that occurs usually in the normal intestinal flora. It employs various survival strategies as an inhabitant of the human intestinal tract, some of which may be beneficial while others may cause harm to the host.[69] They are abundantly increased in LPS-induced intestinal inflammation,[70] Zhu et al reported that omega-3 polyunsaturated fatty acids increased serum IL-10, decreased serum IL-1β, TNF-α , and decreased the relative abundance of *Bacteroides*, so potentially improving the gut microbiota and immunity.[71] Wu et al showed that lauric acid (LA), as a primary MCFA, improved broiler immune function, as evidenced by upregulated immunoglobulins (IgA, IgM, and IgY), downregulated inflammatory cytokines (IL-1β, IL-6, TNF-α, IL-4, and IL-10), and reduced relative abundance of *Bacteroides*.[24] Similarly, our findings that the relative abundance of *Bacteroides* was reduced after MCT intervention and negatively correlated with serum C3, suggest that this might have improved the intestinal immuno-inflammatory status of tunnel workers. Bacteroides is a predominant genus in the human intestine, accounts for almost 25% of the total intestinal microbiota.[69] In our study, since *Bacteroides* is dominant bacterial population, and more abundant in the altered flora, it is potentially of anti-inflammatory value, countering the pro-inflammatory effects of other non-dominant bacteria such as *Roseburia*. In the event, the MCT intervention had favorable effects to the gut microbiota and immune status of tunnel workers.

Pearson's correlation analysis of the other genus-level flora differences and changes of immunoinflammatory indicators with MCT intervention found for Collinsella, Anaerostipes, and Dorea were also anti-inflammatory, while Tyzzerella_3, Megasphaera, Paraprevotella, Coprococcus_2, and Lachnospiraceae_UCG-004 were pro-inflammatory. These findings are not necessarily concordant with those of others.[60,72,73] Conflict may relate to differences in genus attribution, or depth of species or strain recognition. There may be great difference in microbiomic composition at the species level for the same genus, and even nonbacterial kingdom such as that of virus, fungus or archaea, with beneficial or adverse effect on human immunity.[33]

***Milk consumption and gut microbiomics***

Since the same non-fermented milk product was used as the matrix of both the intervention and placebo in this study, the milk itself will have had an impact on the gut microbiota. Partula et al. found that habitual milk intake was positively associated with Streptococcus in the colon,[74] Shuai et al found that some butyrate-producing genera, such as *Clostridium*, *Roseburia* and *Lachnobacterium*, can even serve as the biomarkers of high dairy intake.[75] Butyrate has been shown to improve the gut microbiota and immunity.[76] However, in this study, no significant changes at the genus level were found in the control group before and after the intervention, which may be on account of limited sample size. In addition, lactose intolerant workers were excluded from this study to avoid the potential generation of diarrheal symptoms seen with higher dose lactose on the primary experimental outcome measure (incidence of diarrhea), and to minimize the risk

of adverse events. However, even some so-called lactose intolerant people will not experience diarrhea when they consume no more than 25g of lactose (about 500mL of milk product).[77] Moreover, undigested lactose can also be fermented in the colon to produce SCFAs and alter the gut microbiota, thereby affecting immune status.[78] Nevertheless, the possible inclusion of lactose non-digesters, who can tolerate higher lactose intakes, in our study may complicate the interpretation of our findings. However, we maximized the reliability of any difference between the two groups on the basis of MCT intake with similar food systems for the two groups for background dietary and dairy intakes. However, differences in gut microbiota before and after MCT intervention need confirmation in by studies with expanded sample size.

### *Strengths and limitations*

The strength of our study is its double-blinded and randomized design. Furthermore, the workers diet, physical activity, and living environment were similar, largely eliminating external confounding factors between the two groups. However, our study has certain limitations. Firstly, the small sample size limits statistical power and presents challenges to its generalization. Secondly, we only detected taxa at the genus level by 16S rRNA sequencing analysis, which may overlook possible associations at the species or strain levels. Thirdly, although we found that the improved immune status was closely related to changes in the abundance of some genera in the gut, a causal relationship between the two cannot be asserted. Fourthly, the reduction in occurrence of diarrhea was observed within the intervention group, but not by comparison with the reference group, so that this observation must be provisional on further study.

### *Conclusions*

In summary, we observed that consumption of MCT reduced the occurrence of diarrhea, with an associated improvement in immune status and gut microbiota in tunnel workers. The immunoinflammatory factor profile induced by MCT, with an anti-inflammatory trend, was significantly correlated with the changes in gut microbiota at the genus level. However, whether MCT improves immune status by regulating gut microbiota and its mechanism needs more targeted and convincing evidence from further studies in other occupational and vulnerable groups over more extended time frames.

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**AUTHOR DISCLOSURES**

The authors declare no conflict of interest.

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