

Original Article

Change in gut microbiota is correlated with alterations in the surface molecule expression of monocytes after Roux-en-Y gastric bypass surgery in obese type 2 diabetic patients

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Abstract: *Objectives:* Persistent low-grade chronic inflammation is common in type 2 diabetes (T2D) and obesity. To date, the underlying molecular mechanism is not well understood. In this study, we aimed to investigate gut microbiota and the expression of monocyte surface molecules in obese T2D subjects who underwent Roux-en-Y gastric bypass (RYGB) surgery. *Methods:* Twenty-four T2D patients were enrolled. Gut microbiota was assessed by measuring bacterial DNA. The phenotypes and biological functions of monocytes, and the expression of monocyte surface molecules were examined by flow cytometry. *Results:* RYGB led to significant alterations in the phenotypes of monocytes. Moreover, the ability of monocyte migration was significantly decreased after RYGB ($P<0.05$), which was consistent with reduced Chemokine-receptors CCR2 expression of CD14⁺CD16⁺ monocytes ($P<0.05$) and CX3CR1 expression of the three monocytes subsets ($P<0.05$). RYGB also resulted in a shift of gut microbiota in the obese T2D patients. Spearman's rank correlation coefficient showed a link between gut microbiota and monocyte subsets where the increased *Bacteroidetes* was negatively correlated with the variation of CD14^{dim}CD16⁺ monocyte percentage ($r=-0.477$, $P<0.05$). Furthermore, the decreased counts of *Escherichia* were positively correlated with the variation of TNF- α secretion ($r=0.442$, $P<0.05$) and TLR4 ($r=0.425$, $P<0.05$) expression on CD14⁺CD16⁺ monocytes. *Conclusions:* This study, for the first time, demonstrated a link between the changes in gut microbiota and alterations in both phenotypes and functions of monocytes after RYGB, which may contribute significantly to the low-grade chronic inflammation in obese T2D patients.

Keywords: Gut microbiota, type 2 diabetes, monocytes subsets, chronic inflammation, cytokine

Introduction

Low-grade chronic inflammation is characterized by high levels of inflammatory markers such as interleukins, cytokines, and C-reactive protein and is considered one of the major traits in Type 2 diabetes (T2D) and obesity [1]. The molecular mechanism behind T2D-associated chronic inflammation is not well understood. Previous studies have shown that monocytes are important pro-inflammatory effectors of most inflammatory reactions and play a critical role in the initiation of chronic inflammation [2]. Indeed, an increase in the number of monocytes and their activation were found in the adipose tissue (AT) that may contribute to low-

grade chronic inflammation, metabolic syndrome, development of insulin resistance and even pancreatic dysfunction [3]. However, little is known about the trigger that activates of monocytes and initiates chronic inflammation.

In humans, monocytes are classified into at least three subsets based on the different levels of expression of CD14 and CD16 cell surface receptors: the classical monocytes (CM) with only high levels of CD14 (CD14⁺⁺CD16⁻), intermediate monocytes (IM) expressing high levels of CD14 and low levels of CD16 (CD14⁺⁺CD16⁺), and non-classical monocytes (NCM) with low expression of CD14 and co-expression of the CD16 receptor (CD14^{dim}CD16⁺).

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Table 1. Changes in the clinical characteristics of T2D patients following RYGB

Parameters	Before surgery	At 6 months	P
Age (years)	51.5±9.6		
Gender (female/male)	10/14		
Duration of T2DM, yr	7.8±4.6		
Body mass index	46.3±4.7	38.1±3.1	P<0.05
Glycemia (mmol/L)	6.3±0.7	5.1±0.6	P<0.05
HbA1c (%)	7.93±1.55	5.75±1.42	P<0.05
Total cholesterol (mmol/L)	4.93±0.35	4.25±0.44	P<0.05
HDL-C (mmol/L)	1.06±0.06	1.25±0.07	P<0.05
Triglyceride (mmol/L)	1.86±0.08	1.43±0.07	P<0.05
Free fatty acids (mmol/L)	0.69±0.19	0.52±0.17	P<0.05
CRP (mg/L)	10.9±1.2	5.5±1.1	P<0.05

Chemokine-receptors (CCR2, CCR5, and CX3CR1) and cell-surface molecules were also differentially expressed in the three monocytes subsets. IM shows elevated expression of tumor necrosis factor- α (TNF- α), human leukocyte antigen-antigen D related (HLA-DR) proteins, and is a significant producer of pro-inflammatory cytokines. NM exhibits a behavioral crawling on the endothelium in inflammation or damage [4]. It has been reported that the number of CD14^{dim}CD16⁺ monocytes was significantly elevated in T2D patients and was positively correlated with insulin resistance and body mass index (BMI) [5]. The IM plays an important role in many inflammatory diseases including T2D, obesity, sepsis, rheumatoid arthritis, etc [5, 6]. Based on these observations, monocytes subsets were thought to contribute to low-grade chronic inflammation in T2D patients.

Recently, the link between the gut microbiota and innate immunity was discovered in T2D patients. Alterations in the composition of gut microbiota were found to be correlated to low-grade inflammation in T2D patients [7]. The Cell wall components of gut microbiota were associated with the significantly lower methylation of Toll-like receptor 4 (TLR4). Increased penetration of gut membrane impaired by bacterial components was thought to induce this inflammation [8]. *Escherichia* was significantly increased in T2DM patients and was positively correlated with monocyte chemoattractant Protein1 (MCP-1) suggesting that lipopolysaccharides (LPS), a major component of the Gram-negative cell wall, may contribute to the

low-grade inflammation [9]. However, the link between gut microbiota and circulating monocytes subsets in T2D patients has not been well studied.

In the present study, we investigated the hypothesis that an alteration in gut microbiota in obese T2D subjects could be responsible for the low-grade chronic inflammation via the activation of monocytes. We sought to compare the alterations in gut microbiota and the expression of CCR2, CX3CR1, TNF- α , interleukin-6 (IL-6) and TLR4 on monocytes subsets in obese T2D subjects following Roux-en-Y gastric bypass (RYGB) surgery. RYGB is a treatment option for obese T2D patients and a good model to better understand the pathogenesis involved in obese T2D [10]. Subsequently, the correlation between the variation in gut microbiota and the alteration in the cell-surface molecule expression on monocytes subsets was determined. Finally, the goal of this study is to help better understanding the contribution of gut microbiota in the pathogenesis of obese T2D.

Materials and methods

Study participants

Twenty-four adult subjects with T2D (BMI \geq 40 kg/m²) were recruited in this study. All patients were hospitalized at the Department of Hepatobiliary and Pancreatic Surgery of the First Affiliated Hospital of Soochow University in Suzhou, China between November 2013 and March 2016, and were scheduled to undergo RYGB surgery using a surgical technique as reported previously [11]. A minimum of three months of T2D therapy was required for inclusion, while subjects were excluded from this study if they were taking immune suppressing medication. After an overnight fasting, 7 mL of heparinized blood and fresh fecal samples were collected in the morning of the RYGB procedure and 180 days after the surgery. Demographic and clinical characteristics of the subjects were presented in **Table 1**.

All the patients had given a written informed consent. The study was approved (EC/2013/004) by the Ethics Committee of the First Affiliated Hospital of Soochow University.

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Table 2. Primers used for polymerase chain reaction

	F:	R:
<i>Firmicutes</i> phyla	GTCAGCTCGTGTCTGTA	GTCAANTCATCATGCC
<i>Bacteroidetes</i> phyla	GAGAGGAAGGTCCCCAC	CGCTACTTGGCTGGTTCAG
<i>Lactobacillus</i>	AGCAGTAGGGAATCTCCA	CACCGCTACACATGGAG
<i>Bifidobacterium</i>	GCGTGCTTAACACATGCAAGTC	CACCCGTTTCCAGGAGCTATT
<i>Enterococcus</i>	5'CCCTTATTGTTAGTTGCCATCATT3'	5'ACTCGTTGTACTTCCCATTGT3'
<i>Escherichia</i>	5'GTTAATACCTTTGCTCATTGA3'	5'ACCAGGGTATCTAATCCTGTT3'

Flow cytometric analysis of cell-surface molecules and intracellular cytokines of monocytes

100 μ L of peripheral blood was incubated for 30 min with 10 μ L of FITC-labeled CD14 antibody (Beckman Coulter, Brea, CA), 10 μ L of PerCP-Cy5.5-labeled CD16 antibody (Becton Dickinson, Franklin Lake, NJ), 10 μ L of PE-labeled CCR2 antibody (BD Biosciences, San Jose, CA), APC-labeled CX3CR1 antibody (BD Biosciences, San Jose, CA) or 10 μ L of isotype control antibody. Subsequently, the red blood cells were lysed using FACS™ lysing solution (BD Biosciences, San Jose, CA).

For intracellular TNF- α and IL-6 staining, 100 μ L of peripheral blood was incubated with 50 ng/mL Phorbol-12-Myristate-13-Acetate (PMA), 1 μ g/mL ionomycin, and 1 μ g/mL brefeldin (all from Sigma) for 4 h. Subsequently, the red blood cells were lysed using FACS™ lysing solution. The cells were then labeled with FITC-labeled CD14 antibody (10 μ L) and PerCP-Cy5.5-labeled CD16 antibody (10 μ L). Next, the cells were fixed using Fixation/Permeabilization and labeled intracellularly with 10 μ L of PE-labeled antibodies directed against TNF- α and 10 μ L of APC-labeled IL-6 antibody (all from BD Biosciences, San Jose, CA). Flow cytometric analysis was performed on a FACSCalibur (BD Biosciences) flow cytometer.

Analysis of monocyte migration

To test migration capacity of monocytes, peripheral blood mononuclear cells (PBMCs) were separated from the blood using lymphocyte separation medium (Hengxin Co., Shanghai, China). PBMCs were seeded and cultured in 24-well plates. 100 μ L aliquots of suspension containing 5×10^5 cells/mL were placed in the upper wells. 100 ng/mL MCP-1, 100 ng/mL IP-10, 1 ng/mL RANTES and a chemokine-mix

(50 ng/mL MCP-1, 50 ng/mL IP-10 and 0.5 ng/mL RANTES) (all obtained from Pepro Tech, Hamburg, Germany) were placed in the lower wells of the chemotaxis chamber. Monocytes in the lower chamber were quantified after 150 minutes at 37°C. Chemotactic index is expressed as increased fold of monocyte response to chemokines compared to medium which was set as one.

Extraction of bacterial DNA and real-time quantitative RT-PCR

200 mg of fresh fecal samples was suspended in a 2 mL of sterile phosphate-buffered saline (PBS) and centrifuged at 200 \times g for 5 min. The supernatant was further centrifuged at 900 \times g for 5 min and then frozen at -20°C. 0.05 g of each standard strain (the General Microbiological Culture Collection Center, China) and total bacterial DNA was extracted from the frozen fecal samples using the bacterial genomic DNA extraction kit (KeyGEN, Nanjing, China) according to the manufacturer's protocol.

16S rDNA of the standard strains was amplified by polymerase chain reaction (PCR) with Premix Taq™ Hot Start Version (TaKaRa, Dalian, China). Primers were showed in **Table 2**. PCR products were purified with MiniBEST DNA Fragment Purification Kit and ligated into the pMD18-T Vector (all from TaKaRa, Dalian, China). The right inserted fragments in plasmids were confirmed by sequencing and the concentration was determined with a NanoDrop 1000 spectrophotometer (Thermo Scientific, Saveen Werner ApS, Denmark). Quantification of bacteria in the fecal samples was determined by real-time quantitative PCR (RT-qPCR) with SYBR Premix Ex Taq™ II (TaKaRa, Dalian, China). The thermal cycler conditions were set as follows: 1 min at 95°C, followed by 40 cycles at 95°C for 15 sec, and 60°C for 1 min.

Statistical analysis

Statistical analysis was performed with Stata 7.0 (StataCorp, College Station, TX). The differences in non-parametric data were expressed as median (M, 25-75 percentile) and analyzed by a two-tailed Mann-Whitney U test. The normal distribution of data was presented as Mean \pm SD ($\bar{X} \pm S$) and statistical significance between two groups were performed using student's t-test. Linear correlation was calculated by Spearman coefficients. $P < 0.05$ was considered statistically significant.

Results

RYGB alters the distribution of monocyte phenotypes and the expression of proinflammatory cytokines in monocytes

We determined the percentages of monocyte subsets, expression of toll-like receptor 2 (TLR2) and TLR4, and intracellular levels of TNF- α and IL-6 of monocytes by measuring median fluorescence intensity (MFI) using flow cytometry. Our results showed an alteration in the distribution of monocyte subsets 6 months after RYGB versus before RYGB, where the percentages of both NCM (CD14^{dim}CD16⁺) and IM (CD14⁺CD16⁺) in peripheral blood were significantly reduced ($P < 0.05$ **Figure 1C, 1D**), and that of CM significantly increased ($P < 0.05$, **Figure 1E**). We also found that RYGB resulted in a significant decrease in the intracellular levels of TNF- α of IM (CD14⁺CD16⁺) ($P < 0.05$, **Figure 1F**), and IL-6 of CM (CD14⁺CD16⁻) and IM (CD14⁺CD16⁺) ($P < 0.05$, **Figure 1G**). In addition, the expression of TLR-4, the receptor for LPS was significantly declined on the surface of CM (CD14⁺CD16⁻) and IM (CD14⁺CD16⁺) (**Figure 2A, 2B**, $P < 0.05$). In contrast, the expression of TLR2, the receptor for peptidoglycans and lipopeptides, was not altered (**Figure 2C, 2D**, $P > 0.05$).

RYGB modulates the monocyte function assessed by in vitro migration

Migration of monocytes from the bloodstream to the inflammatory sites is required for inflammation response, in which chemokines and their receptors are essential for selectively recruiting monocytes and inducing chemotaxis. Therefore, we assessed the expression of a number of chemokine receptors including

CCR2 and CX3CR1 in the distinct monocyte subsets by flow cytometry. CCR2 expression in IM (CD14⁺CD16⁺) (**Figure 3A**, $P < 0.05$) and CX3CR1 expression in all the three monocytes subsets were significantly decreased (**Figure 3B**, $P < 0.05$).

Chemokines MCP-1, RANTES, and IP-10 have been previously demonstrated to be increased in adipose tissue of obese subjects [12]. Next, we included a combination of MCP-1, RANTES, and IP-10 or chemokinemix in our study. The ability of monocyte migration towards the chemokines MCP-1, RANTES, and chemokine-mix (**Figure 3C-F**, $P < 0.05$) were significantly decreased. However, no significant difference was found for the ability of monocyte migration towards the chemokine IP-10 (**Figure 3D**, $P > 0.05$).

Changes in gut bacterial population after RYGB

Next, we examined microbial components before and 6 months after RYGB surgery was undertaken. *Firmicutes* and *Bacteroidetes* phyla constitute about 90% of all intestinal gut bacteria [13]. As shown in **Figure 4**, the abundance of intestinal *Bacteroidetes* phyla was significantly increased after RYGB (**Figure 4B**, $P < 0.05$), whereas the abundance of intestinal *Firmicutes* phyla showed no significant difference (**Figure 4A**, $P > 0.05$).

Although *Bifidobacterium* is not predominating in the gut bacteria, it seems to play a pivotal role in host metabolism [14]. We observed a significant increase in the numbers of *Bifidobacterium* after RYGB (**Figure 4D**, $P < 0.05$). In addition, the numbers of *Escherichia* after RYGB were observed (**Figure 4F**, $P < 0.05$). However, there was no significant difference in *Lactobacillus* and *Enterococcus* counts (**Figure 4C, 4E**, $P > 0.05$).

Correlation between gut bacterial population and monocytes

Built upon the above results, we next investigated whether the change in gut microbiota was correlated with alterations in the expression of surface molecules in monocytes by a Spearman correlation analysis. As shown in **Figure 5**, variation in *Bacteroidetes* was negatively correlated with the decrease in the percentages of NCM (CD14^{dim}CD16⁺) (**Figure 5A**,

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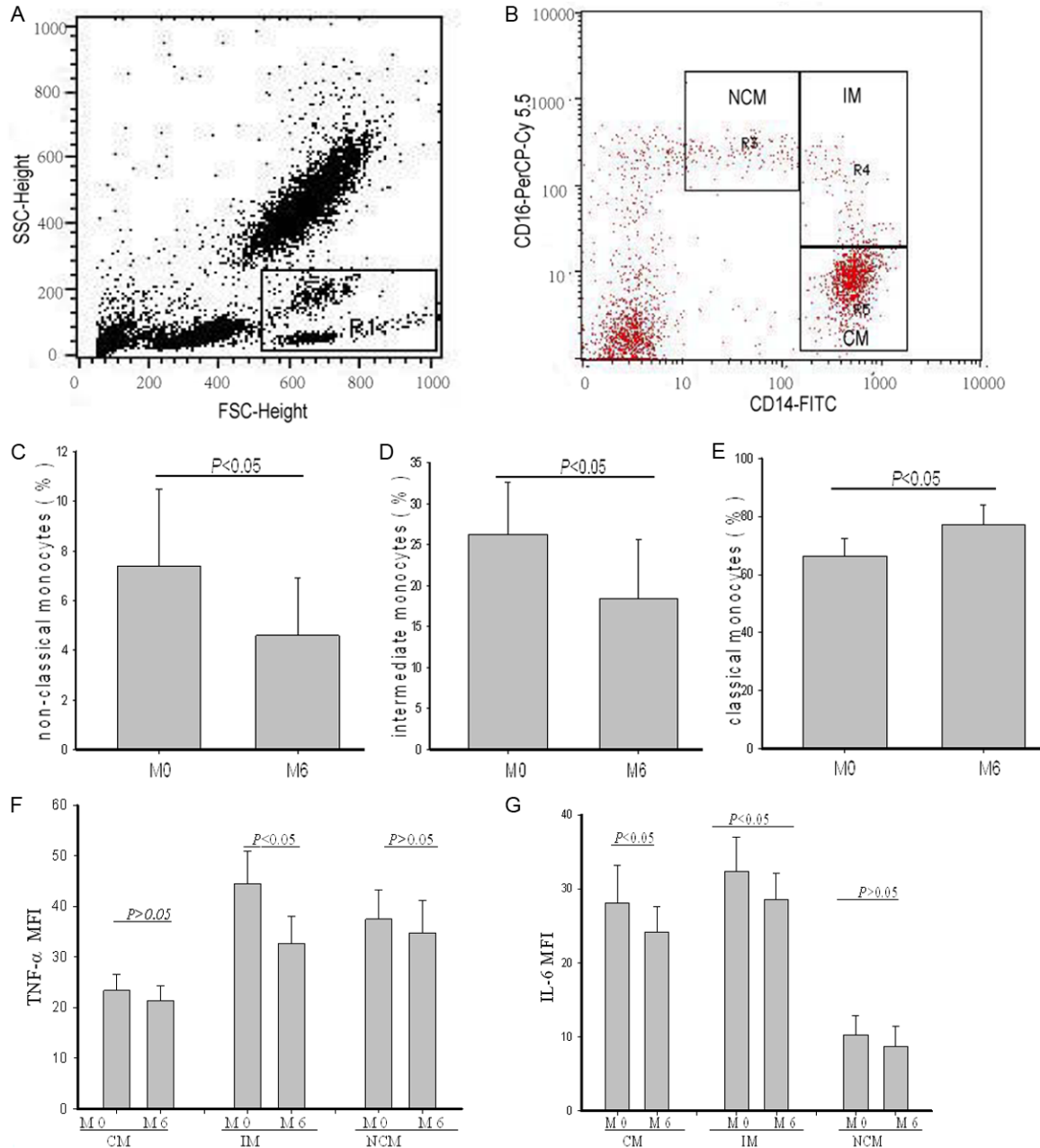


Figure 1. Monocyte subset distribution and intracellular cytokines in patients before and after RYGB. Peripheral blood samples from the recruited patients before and 6 months after RYGB were analyzed and compared for monocyte subset distribution and intracellular cytokines by flow cytometry. (A) Monocytes were initially gated based on light forward and side scatter; (B) Plots showing CD14 and CD16 expression on gated monocytes; Percentages of (C) intermediate monocytes (IM), (D) classical monocytes (CM), and (E) non-classical monocytes (NCM) from patients before (M0) and six months after RYGB (M6); (F) Intracellular cytokines TNF- α and (G) IL-6 assessed by MFI in the three monocyte subsets from patients before and after RYGB. The data were presented as Mean \pm SD and statistical significance was performed using the student's t-test.

$P < 0.05$), whereas the increase in *Bifidobacterium* was negatively associated with the alteration in the CCR2 and CX3CR1 expression in IM (CD14⁺CD16⁺) (Figures 5C, 5D, $P < 0.05$).

In addition, the decreased counts of *Escherichia* were positively correlated with the variation in the levels of TNF- α and TLR4 in IM (CD14⁺CD16⁺) (Figure 5E, 5F, $P < 0.05$).

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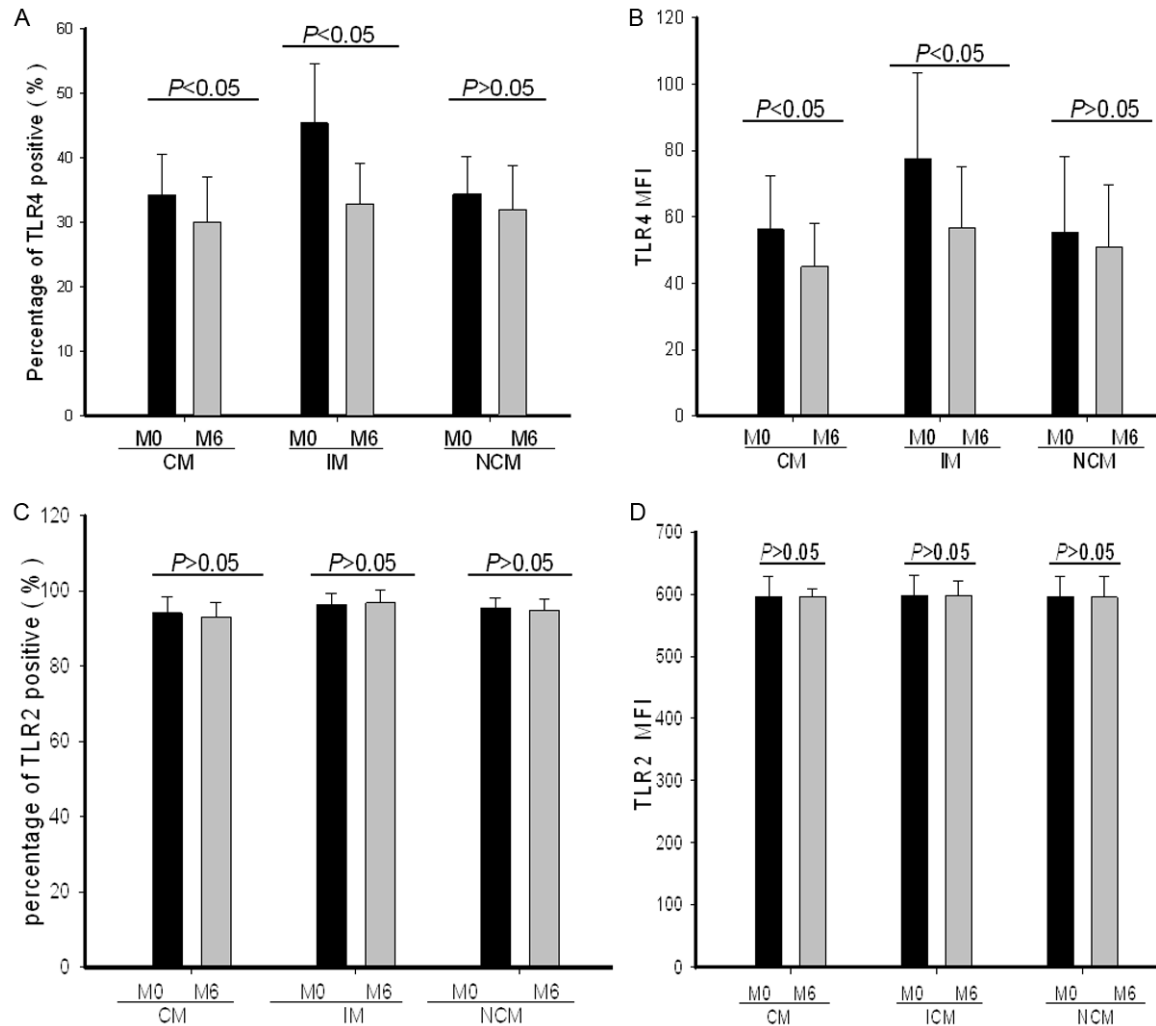


Figure 2. Analysis of TLR4 and TLR2 expression on monocytes subsets. (A) The percentage of TLR4 positive cells and (B) TLR4 assessed by MFI on the three monocytes subsets from the patients before and after RYGB; (C) The percentage of TLR2 positive cells and (D) TLR2 assessed by MFI on monocytes subsets from patients before and after RYGB. Data were presented as Mean \pm SD and statistical significance was performed using the student's t-test.

Discussion

A number of major novel findings in this study were as follows: 1) RYGB significantly affected the phenotypes of monocytes in obese T2D, with a decrease in the inflammatory subpopulations of monocytes; 2) In parallel with the alterations in the phenotypes, the biological functions as assessed by monocyte migration and proinflammatory cytokines of the monocytes subsets were also significantly declined after RYGB in the obese T2D patients; 3) A significant change of gut microbiota after RYGB was detected, mainly with an increase in *Bifidobacterium* but a decrease in *Escherichia*; 4) Spearman's rank correlation coefficient analy-

sis revealed a significant correlation between the shift of gut microbiota and the variations in the frequencies of inflammatory monocyte subsets and cytokines.

In agreement with a previous report, our data showed that RYGB reduced the percentages of both $CD14^{dim}CD16^{+}$ and $CD14^{+}CD16^{+}$ monocytes in T2D subjects [15] as well as the levels of TNF- α and IL-6, the cytokines associated with insulin resistance, in $CD14^{+}CD16^{+}$ monocytes [16, 17]. In addition, we observed a reduction in the expression of TLR-4 on $CD14^{+}CD16^{-}$ and $CD14^{+}CD16^{+}$ monocytes. Previous studies reported that TLR-4, LPS, CRP, MMP-9, and MCP-1 decreased significantly in

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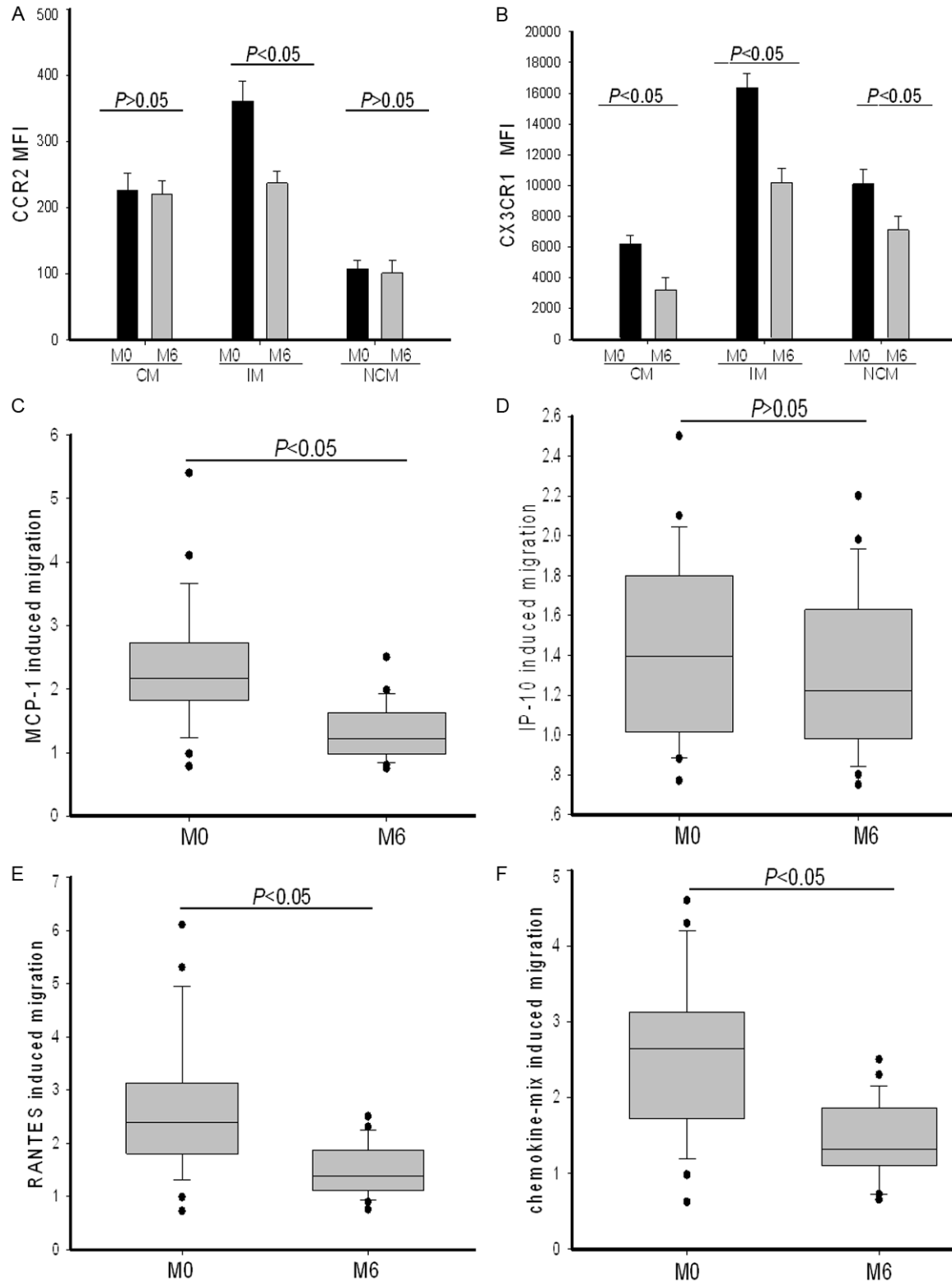


Figure 3. Effects of RYGB on the expression of chemokine receptors and chemokine-induced monocyte migration. (A) CCR2 and (B) CX3CR1 expressions on monocyte subpopulations from patients before and after RYGB. Data was presented as Mean \pm SD and statistical analysis was performed using the student's t-test. Migration capacity of monocytes induced by (C) The chemoattractants MCP-1, (D) IP-10, (E) RANTES, and (F) a chemokine-mix. Box plots represent median (25-75 percentile) and dots represent outliers. Statistical significance was determined by Kruskal-wallis test, and $P < 0.05$ represents statistical significance.

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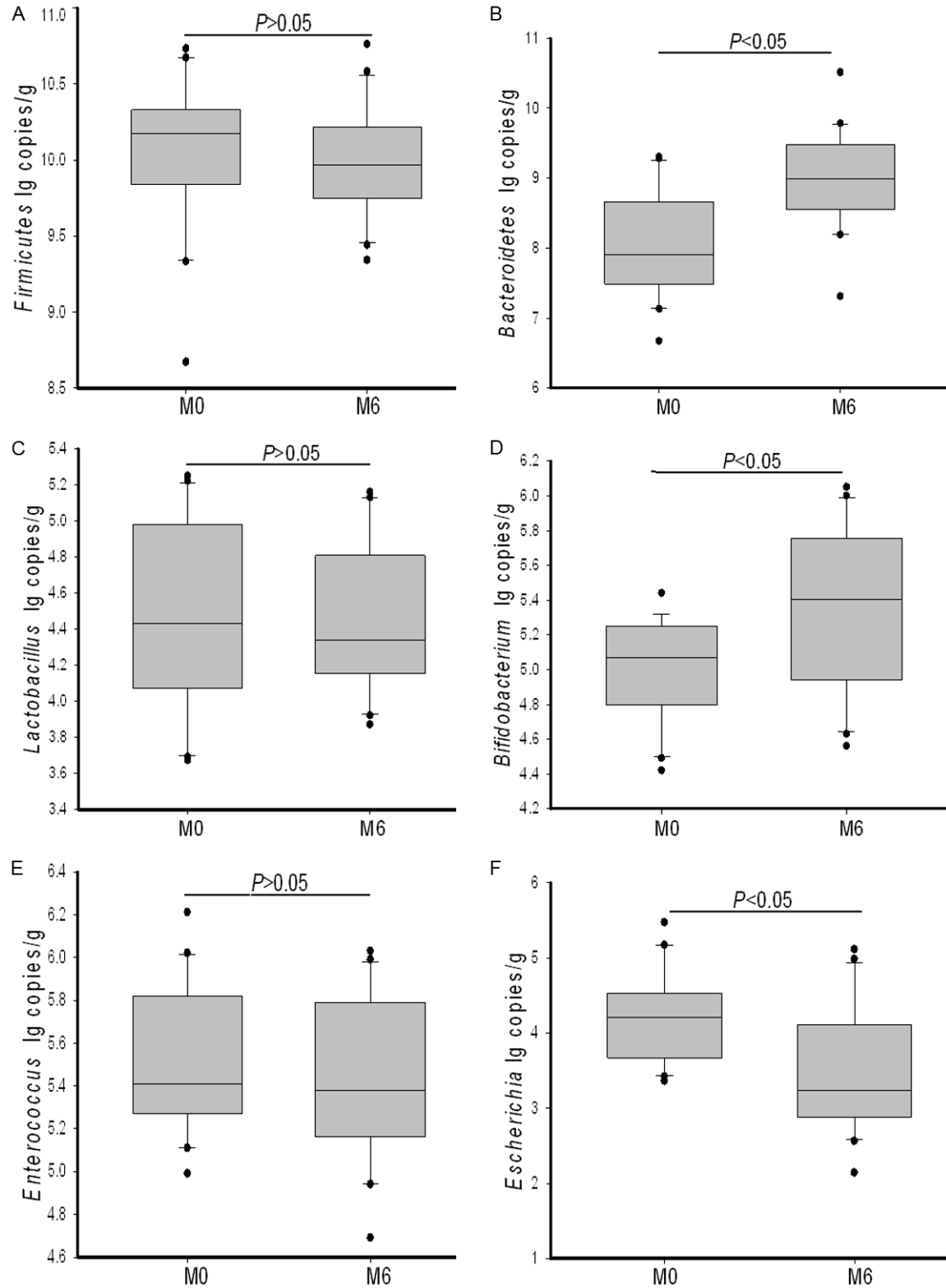


Figure 4. Gut microbiota composition changes after RYGB. The counts of (A) *Firmicutes* phyla, (B) *Bacteroidetes* phyla, (C) *Lactobacillus*, (D) *Bifidobacterium*, (E) *Enterococcus*, and (F) *Escherichia* in fecal samples from patients before (M0) and six months after RYGB (M6). Box plots represent median (25-75 percentile) and dots represent outliers (horizontal line across the middle of the box). Statistical significance was determined by Kruskai-wallis test, and $P < 0.05$ represents statistical significance.

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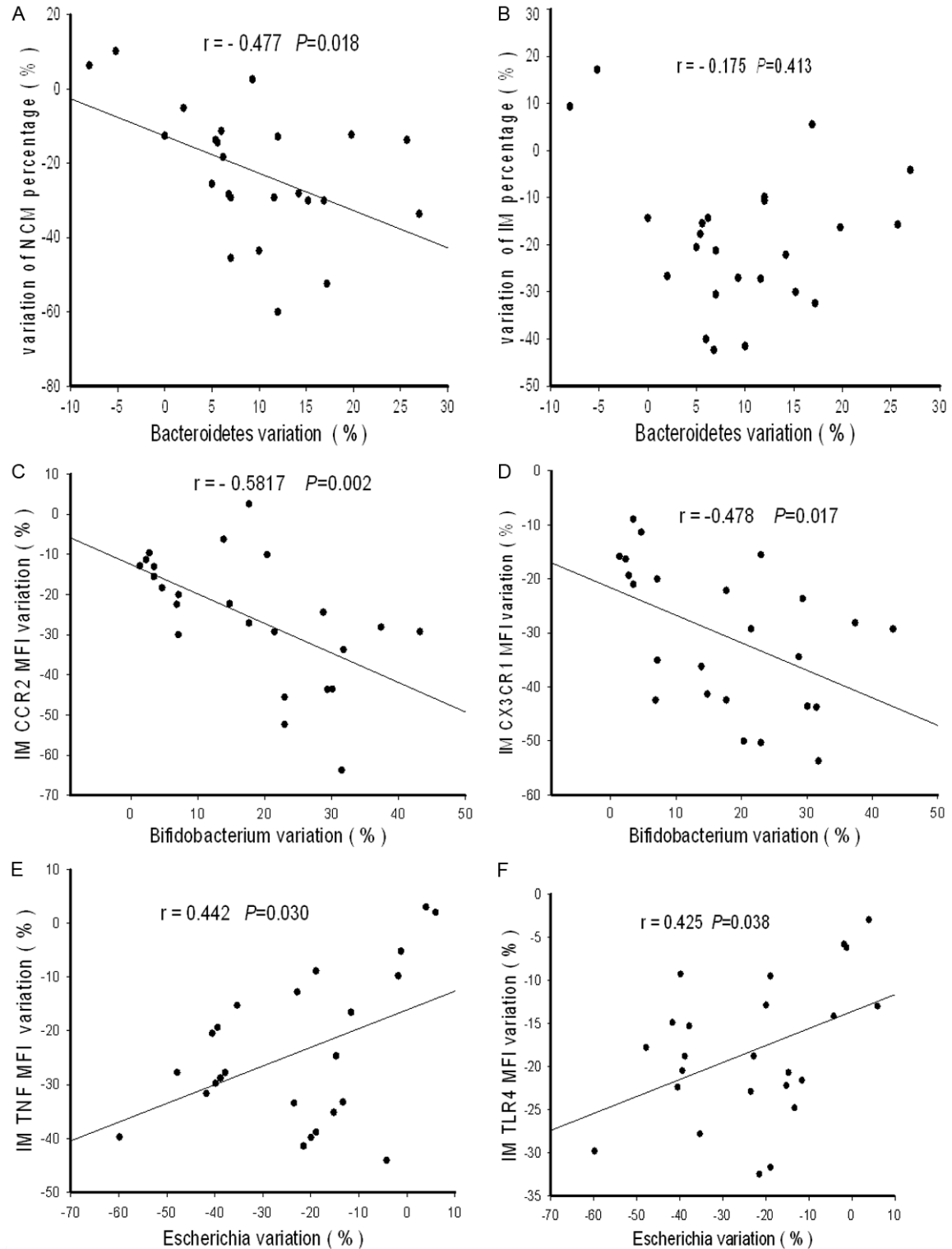


Figure 5. Correlation of gut microbiota composition with monocytes. A, B. Represent correlations between the variation of *bacteroidetes* and the alteration of CD14^{dim}CD16⁺ (NCM) and CD14⁺CD16⁺ (IM) monocytes, respectively, from patients before (M0) and six months after RYGB (M6); C, D. Represent correlations between the variation of *Bifidobacterium* and the changes of CCR2 and CX3CR1 MFI on CD14⁺CD16⁺ monocytes, respectively, from patients before (M0) and six months after RYGB (M6). E, F. Represent correlations between the shifts of *Escherichia* and the variation of TLR4 and TNF- α MFI in CD14⁺CD16⁺ monocytes, respectively, from patients before (M0) and six months after RYGB (M6). “r” denotes the Spearman’s correlation coefficient, and the P value represents statistical significance.

T2D patients after RYGB [18]. Based on these results, RYGB may lead to an anti-inflammatory effect through reducing pro-inflammatory monocyte subsets. The present study also provided the first evidence that RYGB decreases the ability of monocytes migration in obese T2D patients. It has been established that increased infiltration of monocytes within adipose tissue was involved in insulin resistance [19]. For monocyte migration into adipose tissue, it has been thought to be induced by many chemokines and an elevation in the chemokine receptors CCR1, CCR2, CCR3 and CCR5 expression. A significant up-regulation of the CCR2 and CCR5 expression on peripheral monocytes and an elevated chemotactic activity of monocytes collected from T2D and obese subjects were observed, which was associated with insulin resistance [12].

Recent progress in understanding the roles of gut microbiota and inflammation in the pathogenesis of diabetes and obesity has been made. Several studies reported that human obesity was associated with a low abundance of intestinal *Bacteroidetes* phyla and RYGB increased the abundance of intestinal *Bacteroidetes* phyla with a significant reduction in BMI [20]. In addition, RYGB-mediated reduction in the frequency of CD14^{dim}CD16⁺ monocytes was correlated with the variation of BMI in severely obese patients [15]. Built upon these findings, we demonstrated the link between the gut microbiota and monocytes in this study where the increase in numbers of *Bacteroidetes* was negatively correlated with the variation in CD14^{dim}CD16⁺ monocytes. Interestingly, the increase in numbers of *Bifidobacterium* was negatively correlated with CCR2 and CX3CR1 expressions on CD14⁺CD16⁺ monocytes. Previous studies showed that reduced *Bifidobacterium* was negatively correlated with pro-inflammatory cytokines within adipose tissue and these variations can be reversed by weight loss [21, 22]. This is because *Bifidobacterium* improves the function of the mucosal barrier by which the levels of endotoxin is reduced [23]. Furthermore, previous studies have demonstrated that the *Bifidobacterium* counts were strongly correlated with the levels of high-density lipoprotein cholesterol (HDL-C), which, in turn, are positively associated with the CCR2 and CX3CR1 expressions on CD14⁺CD16⁺ monocytes [24, 25]. Thus, the enhancement of

intestinal *Bifidobacterium* may impair the migration ability of CD14⁺CD16⁺ monocytes.

In the present study, our data indicate that the reduction in *Escherichia* counts was positively correlated with the variation in TNF- α and TLR4 levels on CD14⁺CD16⁺ monocytes. Previous reports showed that *Escherichia* counts were elevated in T2D patients suggesting that LPS may be responsible for low-grade inflammation in T2D patients [26, 27]. In addition, Zhu *et al* showed that the protein levels of IL-6 and TNF- α were correlated with TLR4 expression [28]. Because LPS binds to TLR-4 on the surface of monocytes, it is speculated that the decrease in *Escherichia* counts contributed to the reduction in the pro-inflammatory cytokines, including TNF- α and IL-6.

To our knowledge, our study is the first to demonstrate a link between circulating monocytes subsets and gut microbiota in obese T2D patients. Moreover, our findings suggest that the manipulation of the gut microbiome may represent a novel therapeutic approach in diabetic inflammation and thereby may slow down the progression of the T2D. This could be attributed to the effect of large gut bacterial population alterations on changing gut permeability [29]. However, there are some limitations in our study. Although a correlation was observed between the change in gut microbiota and the alterations in phenotypes and biological functions of monocytes, it is difficult to exclude the possibility that the variation in gut microbiota is directly resulted from RYGB or other factors, such as diet, since RYGB reduces preference for high-fat food and total caloric intake, which was associated with the composition of the gut microbiota [30].

In conclusion, the results of our study suggest that alterations of the gut bacterial population are strongly correlated with changes in both phenotypes and biological functions of monocytes, which in turn may contribute to the low-grade chronic inflammation in obese T2D. Thus, modulation of the gut microbiome may represent a novel treatment option for diabetic inflammation. Further investigations are underway in our laboratory to better understand the association between fecal bacteria and host innate immune system in the pathogenesis of T2D.

Acknowledgements

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Disclosure of conflict of interest

None.

Abbreviations

PBMCs, Peripheral blood mononuclear cells; T2D, Type 2 diabetes; IL-6, interleukin-6; TNF- α , tumor necrosis factor- α ; PBS, Phosphate-buffered saline; RYGB, Roux-en-Y gastric bypass; MCP-1, monocyte chemoattractant protein-1; TLR4, Toll-like receptor4; BMI, body mass index; LPS, lipopolysaccharides; MFI, median fluorescence intensity; HDL-C, high density lipoprotein cholesterol.

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