Surgical Endoscopy
https://doi.org/10.1007/s00464-022-09122-z

Laparoscopic sleeve gastrectomy for morbid obesity improves gut microbiota balance, increases colonic mucosal-associated invariant T cells and decreases circulating regulatory T cells

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Received: 11 December 2021 / Accepted: 7 February 2022

Abstract

Background Laparoscopic sleeve gastrectomy (LSG) for morbid obesity may improve gut microbiota balance and decrease chronic inflammation. This study examines the changes in gut microbiota and immune environment, including mucosal-associated invariant T cells (MAIT cells) and regulatory T cells (Treg cells) caused by LSG.

Methods Ten morbidly obese patients underwent LSG at our institution between December 2018 and March 2020. Flow cytometry for Th1/Th2/Th17 cells, Treg cells and MAIT cells in peripheral blood and colonic mucosa and 16S rRNA analysis of gut microbiota were performed preoperatively and then 12 months postoperatively.

Results Twelve months after LSG, the median percent total weight loss was 30.3% and the median percent excess weight loss was 66.9%. According to laboratory data, adiponectin increased, leptin decreased, and chronic inflammation improved after LSG. In the gut microbiota, *Bacteroidetes* and *Fusobacteria* increased after LSG, and indices of alpha diversity increased after LSG. In colonic mucosa, the frequency of MAIT cells increased after LSG. In peripheral blood, the frequency of Th1 cells and effector Treg cells decreased after LSG.

Conclusions After LSG for morbid obesity, improvement in chronic inflammation in obesity is suggested by change in the constituent bacterial species, increase in the diversity of gut microbiota, increase in MAIT cells in the colonic mucosa, and decrease in effector Treg cells in the peripheral blood.

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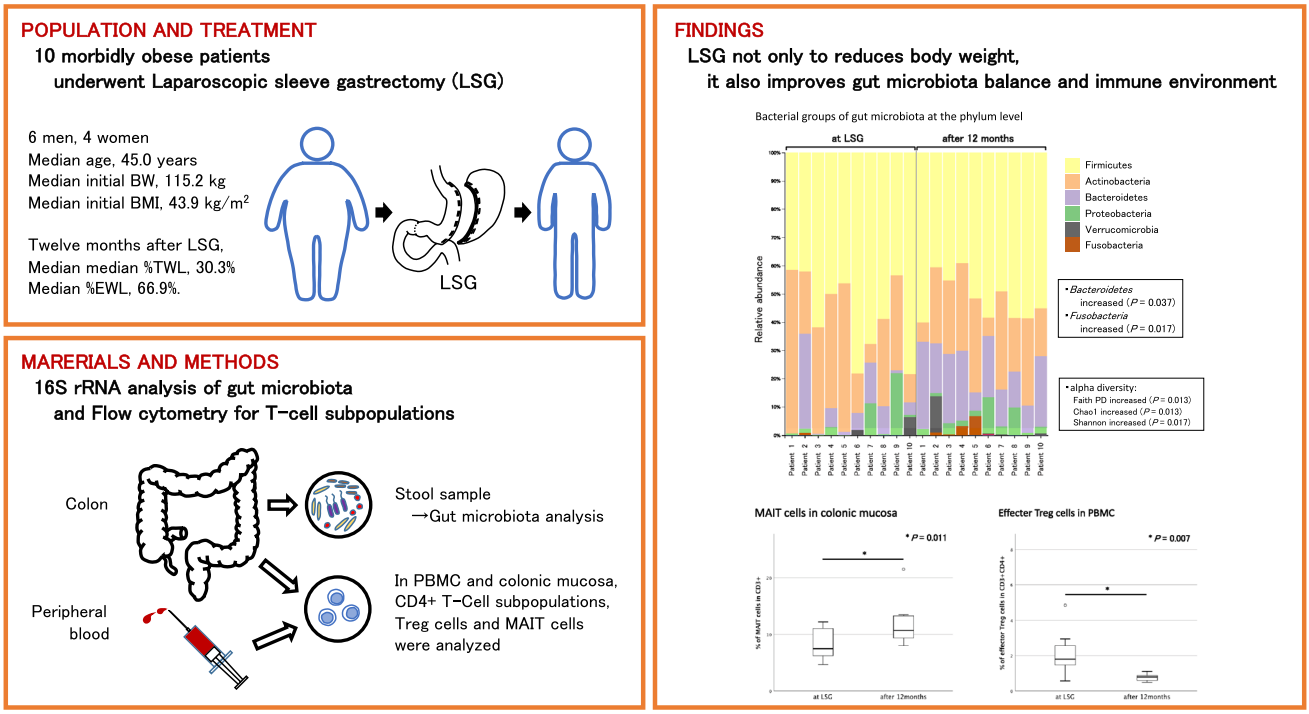
Published online: 19 February 2022

Graphical abstract

Original Article: Laparoscopic sleeve gastrectomy for morbid obesity improves gut microbiota balance, increases colonic mucosal–associated invariant T cells and decreases circulating regulatory T cells



Keywords Laparoscopic sleeve gastrectomy · Bariatric surgery · Metabolic surgery · Gut microbiota · MAIT cell · Treg cell

Morbid obesity has been increasing all over the world, with bariatric surgery highlighted as a treatment option. In Japan, laparoscopic bariatric procedures were first introduced in 2000, followed by introduction of laparoscopic sleeve gastrectomy (LSG) in 2005 [1]; LSG was performed for 704 patients in 2019 [2].

The concept of "metabolic surgery" has rapidly emerged, because bariatric surgery not only reduces body weight (BW) in obesity, it improves various metabolic conditions associated with obesity. Various mechanisms have been reported to improve metabolism after metabolic surgery, including the involvement of gastrointestinal hormones (incretin [3], ghrelin [4], and others), bile acid signaling [5, 6], and adipokines produced by adipocytes (adiponectin, leptin, TNF-α, and others) [7].

Patients with obesity are generally thought to have mild chronic inflammation, which leads to insulin resistance and hyperinsulinemia, and exacerbates various obesity-related comorbidities. Dysfunction of adipose tissue due to excessive accumulation and hypertrophy results in a decrease in anti-inflammatory adipokines, including adiponectin and an increase in inflammatory adipokines such as leptin, TNF-α [8]. Additionally, obesity is known to be a cause of mild inflammation of the intestinal tract, leading to decreased intestinal barrier function and increased blood levels of bacterial endotoxin [9, 10]. Obese patients have also been shown to have changes in the species of gut microbiota and decrease in its diversity, so called dysbiosis, which is thought to be related to chronic inflammation in obesity [11]. The possibility of gut microbiota balance improvement by LSG has not been satisfactorily confirmed.

Gut microbiota is also involved in the establishment of the intestinal immune system, which interacts in a complex manner with host immune function. Components of the intestinal immune system include mucosal-associated invariant T cells (MAIT cells) and regulatory T cells (Treg cells). MAIT cells are innate lymphocytes that are important for the intestinal immune system and are abundant in the lamina propria and Peyer's patches of the intestinal mucosa. In normal-weight and non-metabolically compromised humans, MAIT cells account for about 1–10% of the peripheral blood T cells [12], with an even higher percentage in tissue T cells: reportedly 15–50% in the liver and 10% in the intestines [13, 14]. The antigens of MAIT cells are vitamin B2 metabolic intermediates produced by bacteria [15], and MAIT cells have a protective ability against

bacterial infection by producing inflammatory cytokines and cytotoxic activity [16–18]. Changes in the number of MAIT cells in the colonic mucosa after LSG have not yet been investigated. Treg cells, a type of immunosuppressive cells, express the transcription factor Foxp3 and account for about 5% of the $CD4^+$ T cells in healthy human peripheral blood. In humans, Treg cells mainly exit the thymus into the peripheral blood as naive Treg cells, become effector Treg cells upon antigen stimulation, and regulate immune responses by producing IL-10 and TGF-β [19]. There is a relationship between chronic inflammation and Treg cells [20]. If the patient's chronic inflammatory condition is reduced, there may be changes in the number of Treg cells in peripheral blood. Changes in the number of Treg cells in the peripheral blood after LSG have also not yet been widely investigated.

We hypothesized that obese patients have increased diversity of gut microbiota and improved immune system in intestines and peripheral blood after LSG. As a result, the chronic inflammatory conditions in obese patients may be improved after LSG.

We therefore examined the changes in gut microbiota and the immune environment in the blood and intestines caused by LSG.

Materials and methods

Patients

This single-center prospective study was approved by the Institutional Review Board at the Wakayama Medical University Hospital (WMUH) (Approval Number 2318). All patients gave written informed consent in accordance with its guidelines, and all research has been performed in accordance with the Declaration of Helsinki. The study protocol was registered in the University Hospital Medical Information Network (UMIN000034438).

This study includes ten morbidly obese patients who entered the above clinical trial and underwent LSG at WMUH between December 2018 and March 2020. All patients met the criteria of "(a) body mass index (BMI) $\geq$ 35 kg/m^2" or "(b) BMI $\geq$ 32 kg/m^2 and each have type 2 diabetes mellitus or two or more comorbidities other than type 2 diabetes mellitus (hypertension, dyslipidemia, liver dysfunction, or sleep apnea syndrome)". The criteria for diagnosis in obesity-related comorbidities was based on the criteria "Standardized Outcomes Reporting in Metabolic and Bariatric Surgery" [21].

Operative procedures

Our surgical procedure of LSG was previously reported [22]. Briefly, after setting a 36 Fr bougie tube in the lesser curvature side of the stomach as a guide, sequential firings of linear staplers were performed from 5 cm orad from the pyloric ring to the angle of His. To prevent bleeding and leakage, over-sewing of the staple line was added near the pyloric ring and the angle of His. To prevent torsion, the gastric tube was fixed to the retroperitoneum.

Follow up and data collection

Physical measurement data (height, BW, and BMI), data on obesity-related comorbidities, and laboratory data (blood tests) were obtained on initial visit, at surgery, and then at 1, 3, 6, and 12 months after surgery, and every 3 months thereafter.

Preoperatively (at surgery) and 12 months postoperatively, fat area measurements by plain computed tomography scan were performed, with measurements of hormones and cytokines in the blood (adiponectin, leptin, acyl ghrelin, des-acyl ghrelin).

Preoperatively and 12 months postoperatively, stool samples were collected for gut microbiota analysis, and peripheral blood samples were collected to isolate peripheral blood mononuclear cells (PBMC). In addition, in order to collect lymphocytes in colonic mucosa, colonoscopy was performed, and approximately 12 colonic mucosal tissues were collected with biopsy forceps.

In order to evaluate the weight loss effect, BW, BMI, percent total weight loss (%TWL) and percent excess weight loss (%EWL) were used:

$$\%\text{TWL} = [(\text{initial BW}) - (\text{BW})]/(\text{initial BW}) \times 100$$

$$\%\text{EWL} = [(\text{initial BW}) - (\text{BW})]/[(\text{initial BW}) - (\text{ideal BW})] \times 100$$

$$\text{ideal BW} = \text{height (m)} \times \text{height (m)} \times 25$$

Among obesity-related comorbidities, the criteria for remission and improvement in type 2 diabetes mellitus, dyslipidemia, and hypertension was based on the criteria "Standardized Outcomes Reporting in Metabolic and Bariatric Surgery" [21].

Gut microbiota analysis

Collected stool samples were frozen at – 80 °C for storage. The genomic DNA of the gut microbiota was extracted using the silica-membrane technology with the NucleoSpin Microbial DNA and NucleoSpin Bead Tubes Type B (Takara Bio, Shiga, Japan). Metagenomic analysis of the gut microbiota (16S rRNA analysis [23, 24]) was then performed using MiSeq, a next-generation sequencer (Illumina, San Diego, CA, USA). The sequencing library was constructed by PCR amplification of the V3-V4 region (341F-806R) of 16S rRNA using the 16S (V3-V4) Metagenomic Library Construction Kit for NGS (Takara Bio) and Nextera XT Index

Kit v2 (Illumina). The primers used in the PCR amplification were the 341F primer (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3') and the 806R primer (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAAT-3'). The sequence template formation and the nucleotide sequence acquisition was performed using the MiSeq. Flora analysis was then performed using the Qiime2 pipeline [25]. Operational taxonomy unit (OTU) was first constructed by clustering. Phylogenetic classification was then performed using each OTU based on the information in GreenGenes [26] and DDBJ database [27], and the presence ratio was calculated based on the amount of reads. For each sample, a bar chart was created, and alpha diversity analysis was performed. In the alpha diversity analysis, 100,000 reads were used in each sample, and reads were randomly selected in 10 levels from 1/10th of the total to the total. Rarefaction curves were created for three indices: Faith's Phylogenetic Diversity (Faith PD) [28], Chao1 [29], and Shannon [30]. After confirming that the rarefaction curve had reached a plateau, each index at 100,000 reads was used to compare alpha diversity.

Transition in the number of $CD4^+$ T-Cell subpopulations, Treg cells and MAIT cells

PBMC were separated from the peripheral blood samples by density-gradient centrifugation using a Ficoll-Paque PREMIUM (Cytiva, Tokyo, Japan).

Colonic mucosal tissues were digested in RPMI-1640 with L-Glutamine and Phenol Red (FUJIFILM Wako, Tokyo, Japan) with 10% fetal bovine serum, 1 mg/ml collagenase Type IV and 0.2 mg/ml hyaluronidase Type IV-S at 37 °C for 40 min; 0.5 mg/ml DNase I Type II (Sigma-Aldrich, St. Louis, MO, USA) was then added and the suspension was left at 37 °C for another 20 min. The colonic mucosa suspension was next filtered through a 100-µm nylon mesh. For lymphocyte sorting, $CD45^+$ cells were isolated from the colonic mucosa using CD45 MicroBeads and autoMACS (Miltenyi Biotec, Bergisch Gladbach, Germany).

As $CD4^+$ T-Cell subpopulations, T-helper (Th)1, Th2, and Th17 cells were analyzed. Th1 cells were defined as $CD3^+CD4^+IFN\text{-}\gamma^+$ lymphocytes, Th2 cells as $CD3^+CD4^+IL\text{-}4^+$ lymphocytes, and Th17 cells as $CD3^+CD4^+IL\text{-}17A^+$ lymphocytes. For analysis of Th1/Th2/Th17 cells, PBMC or lymphocytes in colonic mucosa were stimulated with PMA (10 ng/ml) and ionomycin (500 ng/ml) (Sigma-Aldrich) for 5 h in the presence of Brefeldin A (eBioscience, San Diego, CA, USA). Thereafter, the cells were stained with Zombie NIR Fixable Viability Kit, PerCP-Cy5.5-conjugated anti-CD3 and PE-Cy7-conjugated anti-CD4 mAb (BioLegend, San Diego, CA, USA) for 20 min at 4 °C. For intercellular staining, after fixation and permeabilization using Intracellular Fixation & Permeabilization Buffer Kit (eBioscience), the cells were stained with APC-conjugated anti-IFN-γ, Brilliant Violet 421-conjugated anti-IL-4 (BioLegend), and PE-conjugated anti IL-17A mAb (eBioscience) for 20 min at 4 °C.

$FoxP3^+CD4^+$ T cells can be divided into three subpopulations, naive Treg cells, effector Treg cells, and non-Treg cells, depending on the combination of FoxP3 and CD45RA staining [31]. Naive Treg cells were defined here as $CD4^+FoxP3^{lo}CD45RA^+$, effector Treg cells as $CD4^+FoxP3^{hi}CD45RA^-$, and non-Treg cells as $CD4^+FoxP3^{lo}CD45RA^-$. In addition, naive Treg cells + effector Treg cells were designated as Treg cells (All). For analysis of Treg cells, PBMC or lymphocytes in colonic mucosa were stained with Zombie NIR Fixable Viability Kit, PerCP-Cy5.5-conjugated anti-CD3, PE-Cy7-conjugated anti-CD4, and FITC-conjugated anti-CD45RA mAb (BioLegend) for 20 min at 4 °C. For intercellular staining, after fixation and permeabilization using Foxp3 / Transcription Factor Staining Buffer Set (eBioscience), the cells were stained with PE-conjugated anti FoxP3 mAb (eBioscience) for 30 min at 4 °C.

MAIT cells express an invariant T-cell receptor (TCR) alpha chain: Vα7.2-Jα33 in humans and are characterized by high CD161 expression [12, 13], so MAIT cells were defined in this study as $CD3^+CD4^-CD161^{hi}$TCR $V\alpha7.2^+$ lymphocytes. For analysis of MAIT cells, PBMC or lymphocytes in colonic mucosa were stained with Zombie NIR Fixable Viability Kit, PerCP-Cy5.5-conjugated anti-CD3, PE-Cy7-conjugated anti-CD4, Brilliant Violet 510-conjugated anti-CD8, Brilliant Violet 421-conjugated anti-TCR Vα7.2, and FITC-conjugated anti-CD161 mAb (BioLegend) for 20 min at 4 °C.

The cells were analyzed by FACSVerse Flow Cytometer, using FACSuite Software (BD, Franklin Lakes, NJ, USA).

Statistical analysis

All statistical analyses were performed using the SPSS software program, version 25.0 (SPSS Inc., Chicago, IL, USA). Descriptive results regarding continuous variables are reported as median and interquartile range (IQR). Differences were analyzed using the Wilcoxon signed-rank test. A two-sided P value of < 0.05 was considered statistically significant.

Results

Patient characteristics, surgical outcomes and treatment outcomes

The patient characteristics and the surgical outcomes are summarized in Table 1. There were six men and four

women, with a median age 45.0 years. Median initial BW and BMI were 115.2 kg and 43.9 kg/m^2, respectively.

Table 2 shows the treatment outcomes. Twelve months after LSG, the median BW was 89.0 kg, median BMI was 30.6 kg/m^2, median %TWL was 30.3% and median %EWL was 66.9%. According to laboratory data, C-reactive protein (CRP) significantly decreased ($P=0.008$) and albumin significantly increased ($P=0.008$) after LSG. Regarding adipocytokines, adiponectin significantly increased ($P=0.005$) and leptin significantly decreased ($P=0.009$) after LSG. The improvement rate (remission and improvement) of obesity-related comorbidities at 12 months postoperatively was 100% (4/4 cases) for type 2 diabetes mellitus, 42.9% (3/7 cases) for dyslipidemia, and 83.3% (5/6 cases) for hypertension (data not shown).

Table 1 Patient characteristics and surgical outcomes

Variable	Value
Patient characteristics	
Sex (male/female)	6/4
Age, years (median [IQR])	45.0 [35.8–52.0]
Initial BW, kg (median [IQR])	115.2 [97.9–146.9]
Initial BMI, kg/m^2 (median [IQR])	43.9 [38.2–46.5]
Comorbidities, no. (%)	
Type 2 diabetes mellitus	4 (40)
Dyslipidemia	7 (70)
Hypertension	6 (60)
Sleep apnea syndrome	7 (70)
Liver dysfunction	7 (70)
Surgical outcomes	
Operation time, min (median [IQR])	127.5 [115.0–149.3]
Blood loss, ml (median [IQR])	10.0 [8.7–11.3]
Intraoperative and postoperative complications, no. (%)	
All CD grade	1[a] (10)

IQR interquartile range, *BW* body weight, *BMI* body mass index, *CD* Clavien-Dindo classification

[a]Postoperative intra-abdominal bleeding (CD grade I)

Distribution and diversity of gut microbiota

The 16S rRNA analysis of the gut microbiota was performed for ten patients at surgery and 12 months after surgery, and the relative abundances were calculated within phylum level. Figure 1 shows a bar chart of phylum level. The median relative abundances of *Bacteroidetes* was 5.14% (IQR, 0.80–10.96) preoperatively and 19.63% (IQR, 11.95–24.76) 12 months postoperatively, with a significant increase ($P=0.037$). The median relative abundances of *Fusobacteria* was 0.01% (IQR, 0.00–0.03) preoperatively, and 0.20% (IQR, 0.01–1.57) 12 months postoperatively, with a significant increase ($P=0.017$). Otherwise, *Firmicutes* had a median of 53.39% (IQR, 43.03–70.31) vs. a median of 53.28% (IQR, 43.96–58.53) ($P=0.241$), *Actinobacteria* had a median of 32.38% (IQR, 13.10–43.60) vs. a median of 26.68% (IQR, 14.57–31.70) ($P=0.169$), *Proteobacteria* had a median of 0.65% (IQR, 0.14–4.91) vs. a median of 2.27% (IQR, 1.61–5.23) ($P=0.445$), and *Verrucomicrobi*a had a median of 0.00% (IQR, 0.00–0.47) vs a median of 0.00% (IQR, 0.00–0.32) ($P=1.000$), with no significant change between the preoperative period and 12 months postoperatively.

In the alpha diversity analysis, rarefaction curves were made for three indices, Faith PD, Chao1, and Shannon, before and 12 months after LSG (Fig. 2). The median Faith PD was 12.2 (IQR, 8.87–14.5) preoperatively and 14.2

Table 2 Treatment outcomes

Variable	Preoperative (at LSG)	12 months postoperative	*P* value[b]
BW (kg)[a]	106.5 [92.7–131.6]	89.0 [70.2–96.6]	0.005
BMI (kg/m^2)[a]	40.3 [35.5–43.2]	30.6 [26.9–35.0]	0.005
%TWL (%)[a]	7.8 [5.0–9.8]	30.3 [20.8–38.5]	0.005
%EWL (%)[a]	19.1 [12.0–24.3]	66.9 [53.9–89.5]	0.005
Laboratory tests			
CRP (mg/dL)[a]	0.23 [0.09–0.61]	0.07 [0.02–0.13]	0.008
Albumin (g/dL)[a]	4.00 [3.88–4.10]	4.30 [3.90–4.55]	0.008
Adiponectin (μg/mL)[a]	1.64 [0.85–2.92]	4.69 [1.83–8.05]	0.005
Leptin (ng/mL)[a]	32.4 [25.0–40.1]	13.0 [10.3–21.6]	0.009
Acyl ghrelin (fmol/mL)[a]	6.49 [3.88–11.6]	3.05 [0.92–6.03]	0.008
Des-acyl ghrelin (fmol/mL)[a]	19.86 [14.21–35.22]	10.15 [4.36–17.11]	0.021

LSG laparoscopic sleeve gastrectomy, *BW* body weight, *BMI* body mass index, *%TWL* percent total weight loss, *%EWL* percent excess weight loss, *CRP* C-reactive protein, *NS* not significant

[a]Median [interquartile range]

[b]Determined via Wilcoxon signed-rank test

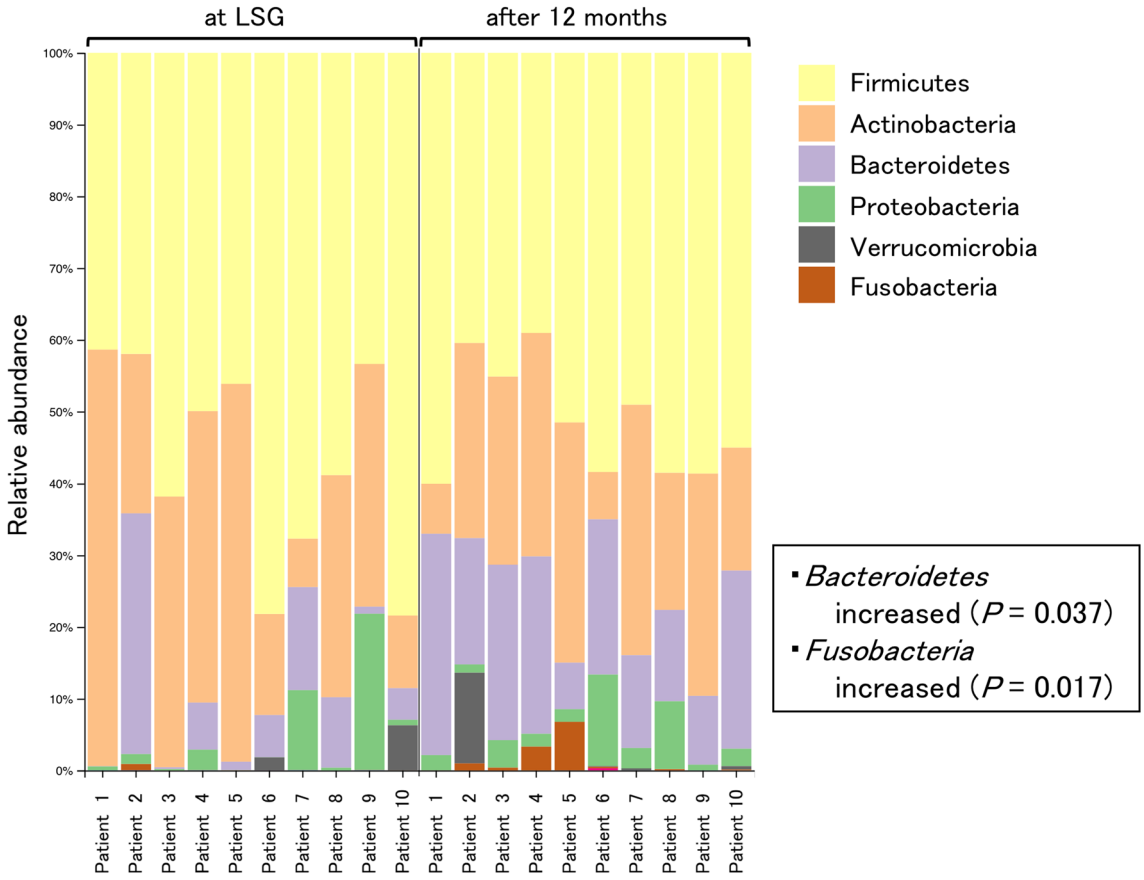


Fig. 1 Relative abundances of bacterial groups at the phylum level. The 16S rRNA analysis of the gut microbiota was performed for ten obese patients at surgery and 12 months after surgery. At the phylum level, the median relative abundances of *Bacteroidetes* and *Fusobacteria* significantly increased between the preoperative period and 12 months postoperatively ($P = 0.037$ and $P = 0.017$)

(IQR, 11.7–20.2) 12 months postoperatively, with a significant increase ($P = 0.013$) (Fig. 2A). The median Chao1 was 175.3 (IQR, 116.9–234.6) preoperatively and 225.5 (IQR, 178.3–379.8) 12 months postoperatively, with a significant increase ($P = 0.013$) (**Fig. 2B**). The median Shannon was 4.56 (IQR, 3.89–5.45) preoperatively and 5.26 (IQR, 4.89–6.14) 12 months postoperatively, with a significant increase ($P = 0.017$) (Fig. 2C).

Transition in the number of Th1/Th2/Th17 cells, Treg cells and MAIT cells

Transition in the number of T-cell subpopulations are showed in Table 3.

In PBMC, the frequency of Th1 cells in $CD3^+CD4^+$ lymphocytes were a median of 33.22% preoperatively and a median of 21.45% 12 months postoperatively, with a significant decrease ($P = 0.009$). The frequency of Th2/Th17 in $CD3^+CD4^+$ lymphocytes were not significantly different between the preoperative period and 12 months postoperatively. In colonic mucosa, the frequency of Th1/Th2/Th17 in $CD3^+CD4^+$ lymphocytes were not significantly different between the preoperative period and 12 months postoperatively.

In PBMC, the frequency of Treg cells (All) in $CD3^+CD4^+$ lymphocytes were a median of 3.62% preoperatively and a median of 2.32% 12 months postoperatively, with a significant decrease ($P = 0.028$) (Fig. 3A). The frequency of naive Treg cells in $CD3^+CD4^+$ lymphocytes were not significantly different between the preoperative period and 12 months postoperatively (Fig. 3B). However, the frequency of effector Treg cells in $CD3^+CD4^+$ lymphocytes were a median of 1.80% preoperatively and a median of 0.79% 12 months postoperatively, with significant decrease ($P = 0.007$) (Fig. 3C). The representative flow cytometry analysis profiles of Treg cells in PBMC are shown in Fig. 3D. In this analysis of 10 patients, the frequency of Treg cells (All) and effector Treg cells in PBMC clearly decreased after LSG. In colonic mucosa, the frequency of Treg cells (All), naive Treg cells and effector Treg cells in $CD3^+CD4^+$ lymphocytes were not significantly different between the preoperative period and 12 months postoperatively (Fig. 3E, F, G).

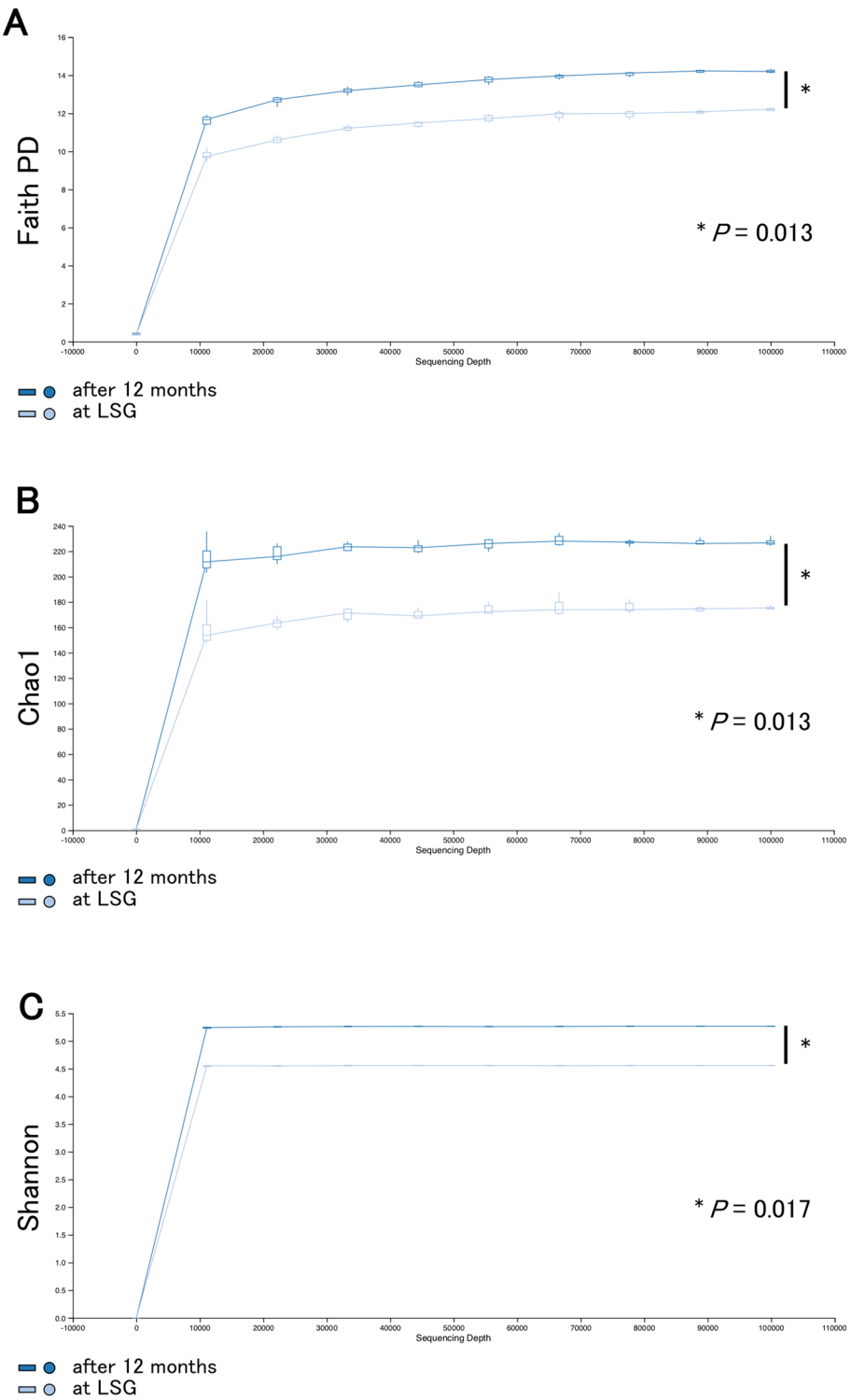


Fig. 2 Rarefaction curve. Each rarefaction curve for the three indices, Faith PD, Chao1, and Shannon, before and 12 months after LSG, had reached a plateau. Each index was compared and examined at 100,000 leads. **A** The median Faith PD significantly increased between the preoperative period and 12 months postoperatively ($P = 0.013$). **B** The median Chao1 significantly increased between the preoperative period and 12 months postoperatively ($P = 0.013$). **C** The median Shannon significantly increased between the preoperative period and 12 months postoperatively ($P = 0.017$)

In PBMC, the frequency of MAIT cells in $CD3^+$ lymphocytes were not significantly different between the preoperative period and 12 months postoperatively (Fig. 4A). In colonic mucosa, the frequency of MAIT cells in $CD3^+$ lymphocytes were a median of 7.48% preoperatively and a median of 10.74% 12 months postoperatively, with a significant increase ($P = 0.011$) (Fig. 4B). The representative flow cytometry analysis profiles of MAIT cells in colonic mucosa is shown in Fig. 4C. In this analysis of ten patients, the frequency of MAIT cells in colonic mucosa clearly increased after LSG.

Table 3 Transition in the number of T-cell subpopulations

Variable	Preoperative (at LSG)	12 months postoperative	*P* value[b]
In PBMC			
$CD4^+$ T-cells, % (in $CD3^+$)[a]	64.74 [55.95–73.89]	70.15 [60.88–78.54]	NS (0.059)
Th1 cells, % (in $CD3^+CD4^+$)[a]	33.22 [22.63–39.65]	21.45 [14.51–25.75]	0.009
Th2 cells, % (in $CD3^+CD4^+$)[a]	2.36 [1.81–4.03]	2.21 [1.31–4.56]	NS (0.721)
Th17 cells, % (in $CD3^+CD4^+$)[a]	3.46 [2.60–5.77]	3.69 [2.79–6.04]	NS (0.799)
Treg cells (All), % (in $CD3^+CD4^+$)[a]	3.62 [2.90–4.87]	2.32 [1.85–2.71]	0.028
naive Treg cells, % (in $CD3^+CD4^+$)[a]	1.68 [1.21–2.25]	1.47 [0.89–1.93]	NS (0.721)
effector Treg cells, % (in $CD3^+CD4^+$)[a]	1.80 [1.41–2.66]	0.79 [0.59–0.88]	0.007
$CD8^+$ T-cells, % (in $CD3^+$)[a]	29.84 [21.23–35.66]	22.03 [18.30–32.99]	NS (0.241)
MAIT cells, % (in $CD3^+$)[a]	1.53 [1.00–2.30]	0.79 [0.43–1.53]	NS (0.139)
In colonic mucosa			
$CD4^+$ T-cells, % (in $CD3^+$)[a]	58.35 [20.33–75.63]	59.07 [52.48–69.09]	NS (0.314)
Th1 cells, % (in $CD3^+CD4^+$)[a]	1.13 [0.88–5.06]	3.57 [2.23–8.46]	NS (0.110)
Th2 cells, % (in $CD3^+CD4^+$)[a]	4.16 [2.95–6.60]	4.99 [3.57–8.62]	NS (0.086)
Th17 cells, % (in $CD3^+CD4^+$)[a]	7.88 [4.37–12.52]	9.29 [5.74–12.92]	NS (0.139)
Treg cells (All), % (in $CD3^+CD4^+$)[a]	5.97 [2.40–7.45]	4.84 [4.25–9.51]	NS (0.441)
naive Treg cells, % (in $CD3^+CD4^+$)[a]	4.07 [1.18–5.91]	3.46 [2.75–5.95]	NS (0.953)
effector Treg cells, % (in $CD3^+CD4^+$)[a]	1.69 [0.57–1.93]	1.84 [1.21–2.84]	NS (0.374)
$CD8^+$ T-cells, % (in $CD3^+$)[a]	35.19 [22.38–42.67]	34.13 [27.81–52.20]	NS (0.214)
MAIT cells, % (in $CD3^+$)[a]	7.48 [5.95–11.47]	10.74 [9.01–13.40]	0.011

LSG laparoscopic sleeve gastrectomy, *PBMC* peripheral blood mononuclear cells, *Treg cells* regulatory T cells, *MAIT cells* mucosal-associated invariant T cells, *NS* not significant

[a]Median [interquartile range]

[b]Determined via Wilcoxon signed-rank test

Discussion

This is the first study to investigate the changes in gut microbiota and various subpopulations of inflammatory cells in both peripheral blood and colonic mucosa after LSG in morbidly obese patients. Our main findings were improvement of gut microbiota balance, increase in the number of MAIT cells in the colonic mucosa, and decrease in the number of Treg cells in the peripheral blood after LSG.

In our data, adiponectin significantly increased and leptin significantly decreased after LSG. In addition, after LSG, CRP significantly decreased and albumin significantly increased, suggesting that chronic inflammation improves after LSG. The accumulation of visceral adipose tissue due to obesity is known to decrease the adiponectin and to induce chronic inflammation, insulin resistance, and atherosclerosis [32]. Meanwhile, adiponectin is known to increase with weight loss after bariatric/metabolic surgery [33]. Leptin is known to act directly on the hypothalamus to transmit suppression signals of food intake, as well as to promote energy expenditure through increased sympathetic nerve activity, thereby inhibiting the development of obesity [34]. In obese patients, leptin production from adipose tissue is known to increase in proportion to body fat mass, and the leptin resistance status is elicited [35]. Leptin has been found to be significantly lower and the leptin resistance status is alleviated after bariatric/metabolic surgery [36]. Changes in adipokines (adiponectin, leptin) by LSG may therefore contribute to the improvement of chronic inflammation, insulin resistance, atherosclerosis, and eventually obesity-related comorbidities.

Obese patients have also been reported to show gut microbiota dysbiosis, and the dysbiosis is leading to decreased intestinal barrier function, increased blood levels of bacterial endotoxin and chronic inflammation in obesity [9–11]. Gut microbiota due to LSG in obese patients was shown in a previous study to increase in *Bacteroidetes* and decrease in *Firmicutes* [37]. In our data, in gut microbiota, LSG significantly increased *Bacteroidetes* and *Fusobacteria* at the phylum level. Furthermore, all three alpha diversity indices increased significantly after LSG, indicating increase in the diversity of gut microbiota. These changes indicate the improvement of gut microbiota balance, and may be related to improvement of chronic inflammation in obesity. Bypass procedures like the laparoscopic Roux-en-Y gastric bypass (LRYGB) or laparoscopic sleeve gastrectomy with duodenojejunal bypass (LSG-DJB) are known to dramatically alter the gut microbiota by modifications in the digestive tract [38], but LSG is also reported alter the gut microbiota [6, 37]. We speculate that the changes in gut microbiota after

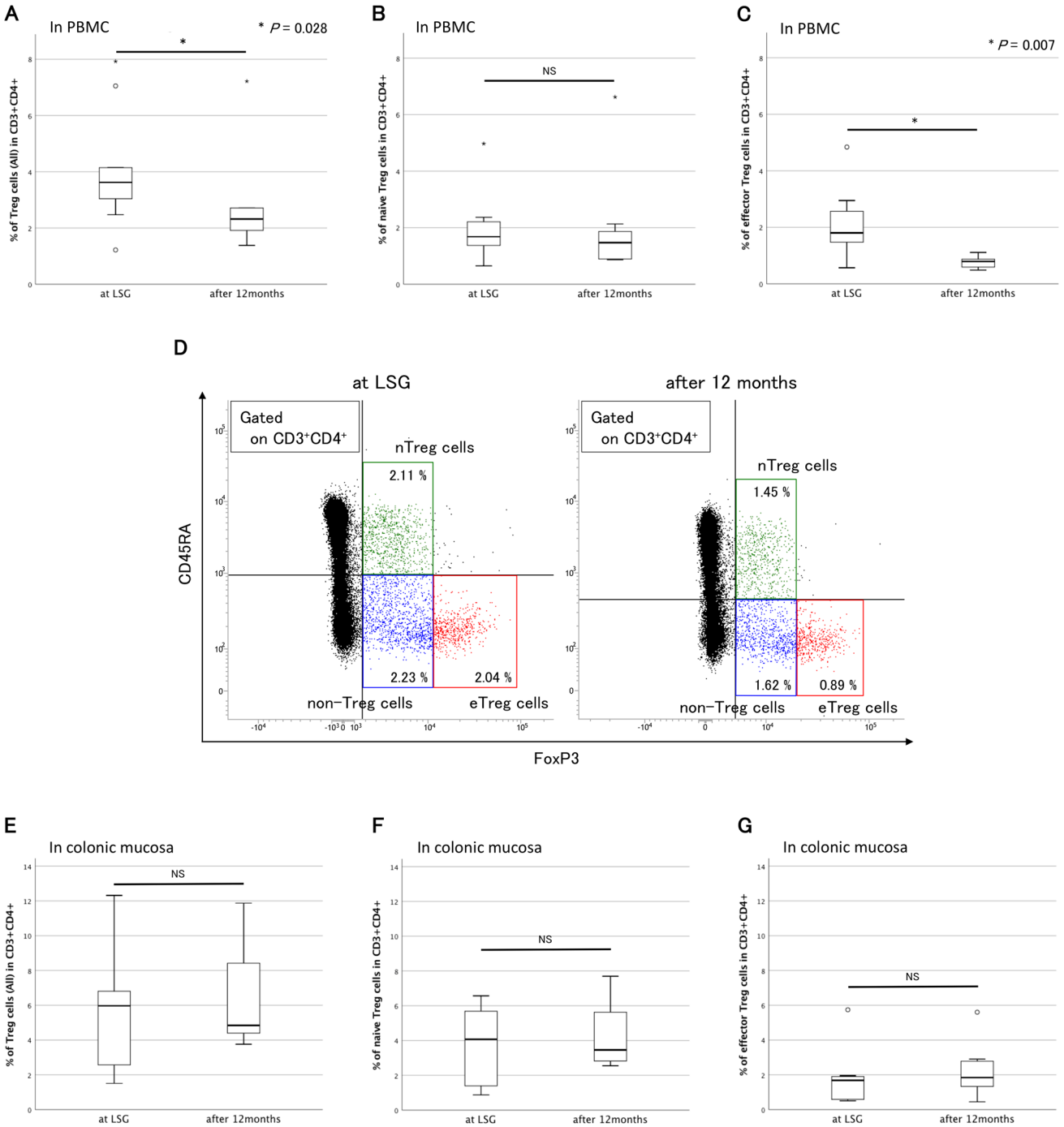


Fig. 3 Transition in the number of Treg cells. **A** In PBMC, the frequency of Treg cells (All) in $CD3^+CD4^+$ lymphocytes were significantly decreased between the preoperative period and 12 months postoperatively ($P=0.028$). **B** In PBMC, the frequency of naive Treg cells in $CD3^+CD4^+$ lymphocytes were not significantly different between the preoperative period and 12 months postoperatively ($P=0.721$). **C** In PBMC, the frequency of effector Treg cells in $CD3^+CD4^+$ lymphocytes were significantly decreased between the preoperative period and 12 months postoperatively ($P=0.007$). **D** Representative flow cytometry analysis profiles of Treg cells in PBMC. Naive Treg cells were defined as $CD4^+FoxP3^{lo}CD45RA^+$, effector Treg were defined as $CD4^+FoxP3^{hi}CD45RA^-$, and non-Treg cells as $CD4^+FoxP3^{lo}CD45RA^-$. Naive Treg cells + effector Treg cells were designated as Treg cells (All). **E** In colonic mucosa, the frequency of Treg cells (All) in $CD3^+CD4^+$ lymphocytes were not significantly different between the preoperative period and 12 months postoperatively ($P=0.441$). **F** In colonic mucosa, the frequency of naive Treg cells in $CD3^+CD4^+$ lymphocytes was not significantly different between the preoperative period and 12 months postoperatively ($P=0.953$). **G** In colonic mucosa, The frequency of effector Treg cells in $CD3^+CD4^+$ lymphocytes were not significantly different between the preoperative period and 12 months postoperatively ($P=0.374$). *nTreg cells*, naive Treg cells; *eTeg cells*, effector Treg cells

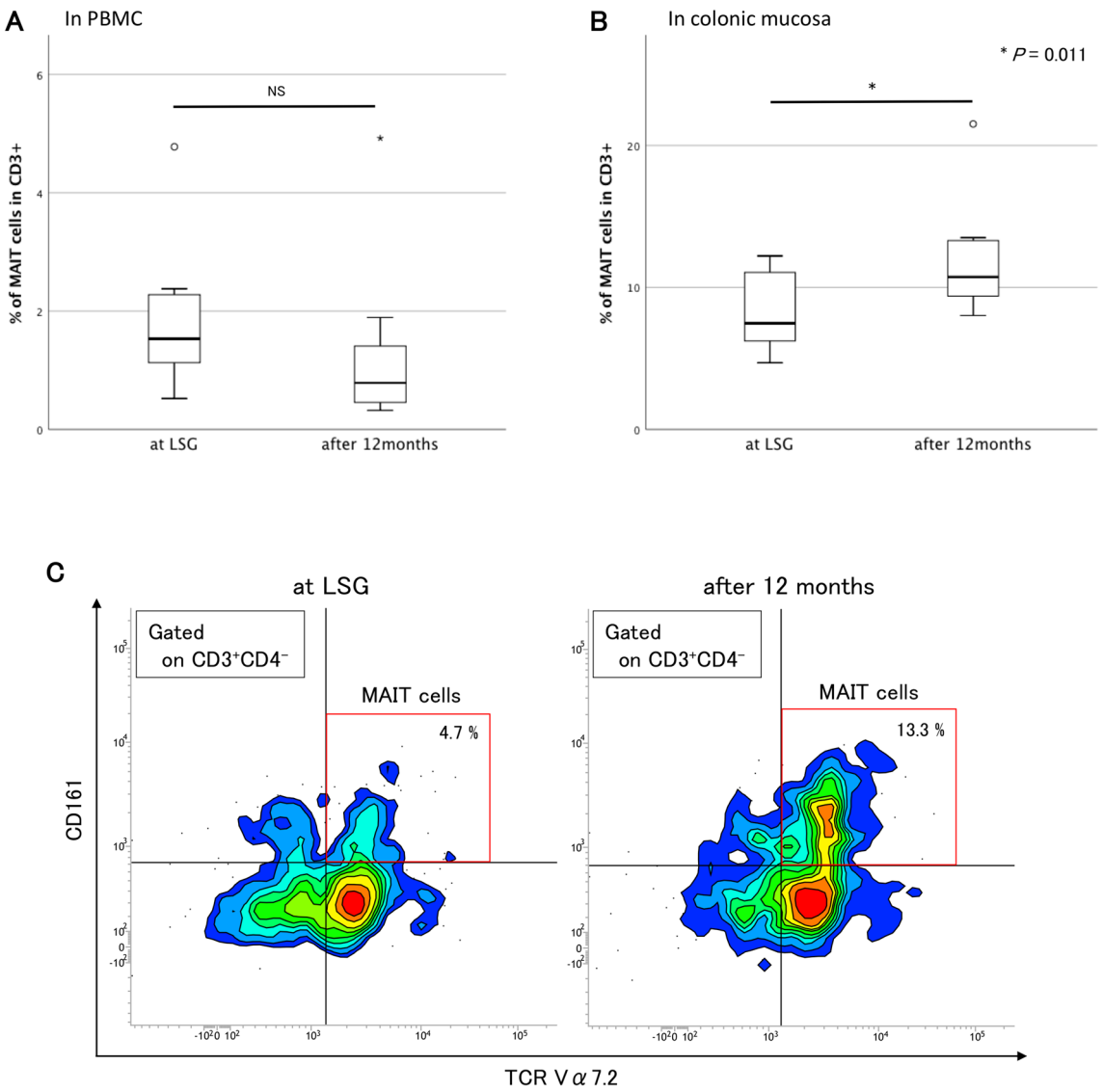


Fig. 4 Transition in the number of MAIT cells. **A** In PBMC, the frequency of MAIT cells in $CD3^+$ lymphocytes were not significantly different between the preoperative period and 12 months postoperatively ($P=0.139$). **B** In colonic mucosa, the frequency of MAIT cells in $CD3^+$ lymphocytes significantly increased between the preoperative period and 12 months postoperatively ($P=0.011$). **C** Representative flow cytometry analysis profiles of MAIT cells in colonic mucosa. MAIT cells were defined as $CD3^+CD4^-CD161^{hi}$TCR $V\alpha7.2^+$ lymphocytes

LSG due to a reduction in diet intake, modifications in the nutrient supply, impairment of bile acid circulation, reduction in gastric juice secretion, and reduction in gastric transit time.

The gut microbiota is also involved in the establishment of the intestinal immune system, and influences MAIT cells and Treg cells. MAIT cells detect bacterial infection using vitamin B2 metabolic intermediates produced by bacteria as antigens [15]. MAIT cells have been reported to be associated with obesity [39, 40]. MAIT cells in adipose tissue and ileum of obese mice are undergoing apoptosis leading to lower frequency [40]. In our data, MAIT cells in colonic mucosa were significantly increased after LSG with increasing of the diversity of gut microbiota.

In our data, *Bacteroidetes* in gut microbiota increased after LSG. *Bacteroides fragilis* has been reported to protect against experimental colitis through the release of polysaccharide A [41]. This anti-inflammatory effect was mediated through the promotion of $CD4^+$ T cells differentiation to $FoxP3^+$ Treg cells in the intestine [42]. Previous reports on Treg cells and obesity have been controversial, as both increases and decreases in Treg cells have been observed in adipose tissue and peripheral blood [19]. A marked reduction of several Treg subpopulations in peripheral blood

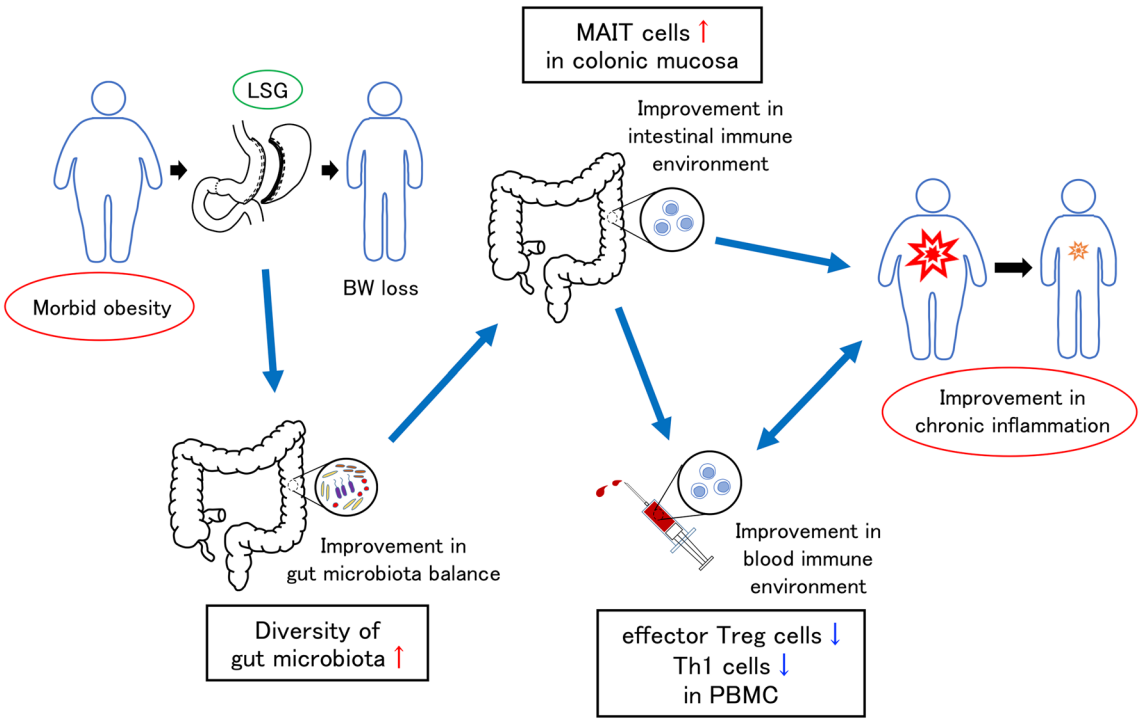


Fig. 5 Speculation about the mechanism for changes caused by the LSG for morbid obesity

was observed in one report in obese patients [43] while another report showed that morbidly obese subjects had a selective increase in circulating Treg cells [44]. In our data, Th1 cells and effector Treg cells in PBMC were significantly decreased after LSG. We speculate that this may be because the chronic inflammation associated with obesity was improved by LSG.

Taken together, we speculate that LSG increased the diversity of gut microbiota and improved the intestinal immune system including MAIT cells, and those things enhanced the intestinal barrier function. This decreased Th1 cells and effector Treg cells in PBMC, leading to the improvement in systemic chronic inflammation in obesity. Figure 5 shows our speculation about the mechanism for changes caused by the LSG for morbid obesity.

This study has several limitations. It was conducted in a single institution with a small number of patients, and there are few obese patients in Japan who require bariatric/metabolic surgery compared with in other countries. The small sample size will likely have decreased statistical power. Because of the small sample size, we did not investigate the data by type of obesity-related comorbidity and the data in obesity without comorbidities. The changes in the diet may be related to gut microbiota, but we did not investigate the diet before and after LSG. The alpha diversity indices increased statistically significantly after LSG, but because of low statistical power, it is unclear whether this make a clinically significant change. Regarding the beta diversity analysis of gut microbiota, we could not show significant results due to low statistical power (data not shown). We speculate that the changes in the constituent bacterial species and the increase in the diversity of gut microbiota after LSG in morbid obese patients may contribute to the improvement of the blood and intestinal immune environment, such as MAIT cells and Treg cells, and thus to the improvement of chronic inflammation in obesity. A multi-center prospective study evaluating benefits including postoperative complications or more long-term outcomes in obese patients treated with LSG is required. Further basic research is also needed in order to prove reduction of chronic inflammation after LSG is required.

Conclusions

After LSG for morbid obesity, improvement in chronic inflammation in obesity is suggested by change in the constituent bacterial species, increase in diversity of gut microbiota, increase in MAIT cells in the colonic mucosa and decrease in effector Treg cells in the peripheral blood.

Acknowledgements We acknowledge proofreading and editing by Benjamin Phillis at the Clinical Study Support Center, Wakayama Medical University. This study was supported by Grant-in-Aid no. 18K16328 and 21K08804 from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and 2020 Wakayama Medical Award for Young Researchers.

Author contributions NF designed the study and wrote the initial draft of the manuscript. NF and TO contributed to data interpretation and critical revision of the manuscript. KH, MK, JK, AT, TG, YU, HI, MN, and HY (all the other nine authors) contributed to data collection and interpretation and critical review of the manuscript. All authors have read and approved the final version of the manuscript and have agreed to be accountable for all aspects of the study, ensuring that any queries related to the accuracy or integrity of any part of the work are answerable.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declarations

Disclosures Drs. Naoki Fukuda, Toshiyasu Ojima, Keiji Hayata, Masahiro Katsuda, Junya Kitadani, Akihiro Takeuchi, Taro Goda, Yoko Ueda, Hiroshi Iwakura, Masahiro Nishi, and Hiroki Yamaue have no conflicts of interest or financial ties to disclose.

Ethical approval This study was approved by the Institutional Review Board at the Wakayama Medical University Hospital (WMUH) (Approval Number 2318).

Informed consent Informed consent was obtained from all patients in accordance with the guidelines of the WMUH Ethics Committee on Human Research.

Human and animal rights All research has been performed in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. The study protocol was registered in the University Hospital Information Network Center in Japan (UMIN000034438).

References

1. Ohta M, Kitano S, Kasama K, Kawamura I, Inamine S, Wakabayashi G, Tani T, Kuwano H, Doki Y, Atomi Y, Kitajima M (2011) Results of a national survey on laparoscopic bariatric surgery in Japan, 2000–2009. Asian J Endosc Surg 4:138–142
2. Ohta M, Kasama K, Sasaki A, Naitoh T, Seki Y, Inamine S, Oshiro T, Doki Y, Seto Y, Hayashi H, Uyama I, Takiguchi S, Kojima K, Mori T, Inomata M, Kitagawa Y, Kitano S, Japan Consortium of Obesity and Metabolic Surgery (JCOMS) (2021) Current status of laparoscopic bariatric/metabolic surgery in Japan: the sixth nationwide survey by the Japan Consortium of Obesity and Metabolic Surgery. Asian J Endosc Surg 14:170–177
3. Laferrère B, Teixeira J, McGinty J, Tran H, Egger JR, Colarusso A, Kovack B, Bawa B, Koshy N, Lee H, Yapp K, Olivan B (2008) Effect of weight loss by gastric bypass surgery versus hypocaloric diet on glucose and incretin levels in patients with type 2 diabetes. J Clin Endocrinol Metab 93:2479–2485
4. Thaler JP, Cummings DE (2009) Minireview: hormonal and metabolic mechanisms of diabetes remission after gastrointestinal surgery. Endocrinology 150:2518–2525
5. Ryan KK, Tremaroli V, Clemmensen C, Kovatcheva-Datchary P, Myronovych A, Karns R, Wilson-Pérez HE, Sandoval DA, Kohli R, Bäckhed F, Seeley RJ (2014) FXR is a molecular target for the effects of vertical sleeve gastrectomy. Nature 509:183–188
6. Ikeda T, Aida M, Yoshida Y, Matsumoto S, Tanaka M, Nakayama J, Nagao Y, Nakata R, Oki E, Akahoshi T, Okano S, Nomura M, Hashizume M, Maehara Y (2020) Alteration in faecal bile acids, gut microbial composition and diversity after laparoscopic sleeve gastrectomy. Br J Surg 107:1673–1685
7. Netto BDM, Bettini SC, Clemente APG, de Carvalho Ferreira JP, Boritza K, de Fátima SS, von der Heyde ME, Earthman CP, Dâmaso AR (2015) Roux-en-Y gastric bypass decreases pro-inflammatory and thrombotic biomarkers in individuals with extreme obesity. Obes Surg 25:1010–1018
8. Ouchi N, Parker JL, Lugus JJ, Walsh K (2011) Adipokines in inflammation and metabolic disease. Nat Rev Immunol 11:85–97
9. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmée E, Cousin B, Sulpice T, Chamontin B, Ferrières J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R (2007) Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 56:1761–1772
10. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R (2008) Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 57:1470–1481
11. Debédat J, Clément K, Aron-Wisnewsky J (2019) Gut microbiota dysbiosis in human obesity: impact of bariatric surgery. Curr Obes Rep 8:229–242
12. Martin E, Treiner E, Duban L, Guerri L, Laude H, Toly C, Premel V, Devys A, Moura IC, Tilloy F, Cherif S, Vera G, Latour S, Soudais C, Lantz O (2009) Stepwise development of MAIT cells in mouse and human. PLoS Biol. https://doi.org/10.1371/journal.pbio.1000054,March10
13. Dusseaux M, Martin E, Serriari N, Pé Guillet I, Premel V, Louis D, Milder M, le Bourhis L, Soudais C, Treiner E, Lantz O (2011) Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. Blood 117:1250–1259
14. Tang X-Z, Jo J, Tan AT, Sandalova E, Chia A, Tan KC, Lee KH, Gehring AJ, de Libero G, Bertoletti A (2013) IL-7 licenses activation of human liver intrasinusoidal mucosal-associated invariant T cells. J Immunol 190:3142–3152
15. Kjer-Nielsen L, Patel O, Corbett AJ, le Nours J, Meehan B, Liu L, Bhati M, Chen Z, Kostenko L, Reantragoon R, Williamson NA, Purcell AW, Dudek NL, McConville MJ, O'Hair RAJ, Khairallah GN, Godfrey DI, Fairlie DP, Rossjohn J, McCluskey J (2012) MR1 presents microbial vitamin B metabolites to MAIT cells. Nature 491:717–723
16. Gold MC, Cerri S, Smyk-Pearson S, Cansler ME, Vogt TM, Delepine J, Winata E, Swarbrick GM, Chua WJ, Yu YYL, Lantz O, Cook MS, Null MD, Jacoby DB, Harriff MJ, Lewinsohn DA, Hansen TH, Lewinsohn DM (2010) Human mucosal associated invariant T cells detect bacterially infected cells. PLoS Biol. https://doi.org/10.1371/journal.pbio.1000407,June29
17. le Bourhis L, Martin E, Péguillet I, Guihot A, Froux N, Coré M, Lévy E, Dusseaux M, Meyssonnier V, Premel V, Ngo C, Riteau B, Duban L, Robert D, Rottman M, Soudais C, Lantz O (2010) Antimicrobial activity of mucosal-associated invariant T cells. Nat Immunol 11:701–708
18. le Bourhis L, Dusseaux M, Bohineust A, Bessoles S, Martin E, Premel V, Coré M, Sleurs D, Serriari NE, Treiner E, Hivroz C, Sansonetti P, Gougeon ML, Soudais C, Lantz O (2013) MAIT cells detect and efficiently lyse bacterially-infected epithelial cells. PLoS Pathog. https://doi.org/10.1371/journal.ppat.1003681,October10
19. Herck MAV, Weyler J, Kwanten WJ, Dirinck EL, Winter BYD, Francque SM, Vonghia L (2019) The differential roles of T cells in non-alcoholic fatty liver disease and obesity. Front Immunol. https://doi.org/10.3389/fimmu.2019.00082,February6
20. Chen X, Wu Y, Wang L (2013) Fat-resident Tregs: an emerging guard protecting from obesity-associated metabolic disorders. Obes Rev 14:568–578

21. Brethauer SA, Kim J, el Chaar M, Papasavas P, Eisenberg D, Rogers A, Ballem N, Kligman M, Kothari S, For the ASMBS Clinical Issues Committee (2015) Standardized outcomes reporting in metabolic and bariatric surgery. Surg Obes Relat Dis 11:489–506
22. Seki Y, Kasama K, Hashimoto K (2016) Long-term outcome of laparoscopic sleeve gastrectomy in morbidly obese Japanese patients. Obes Surg 26:138–145
23. Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC, Schleper C (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442:806–809
24. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI (2009) A core gut microbiome in obese and lean twins. Nature 457:480–484
25. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang K, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857
26. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72:5069–5072
27. Fukuda A, Kodama Y, Mashima J, Fujisawa T, Ogasawara O (2021) DDBJ update: streamlining submission and access of human data. Nucleic Acids Res 49:D71–D75
28. Faith DP (1992) Conservation evaluation and phylogenetic diversity. Biol Cons 61:1–10
29. Chao A (1984) Nonparametric estimation of the number of classes in a population. Scand J Stat 11:265–270
30. Shannon CE (1948) A mathematical theory of communication. Bell Syst Tech J 27(379–423):623–656
31. Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, Parizot C, Taflin C, Heike T, Valeyre D, Mathian A, Nakahata T, Yamaguchi T, Nomura T, Ono M, Amoura Z, Gorochov G, Sakaguchi S (2009) Functional delineation and differentiation dynamics of human $CD4^+$ T cells expressing the FoxP3 transcription factor. Immunity 30:899–911
32. Yamauchi T, Kadowaki T (2013) Adiponectin receptor as a key player in healthy longevity and obesity-related diseases. Cell Metab 17:185–196
33. Herder C, Peltonen M, Svensson PA, Carstensen M, Jacobson P, Roden M, Sjöström L, Carlsson L (2014) Adiponectin and bariatric surgery: associations with diabetes and cardiovascular disease in the Swedish Obese Subjects Study. Diabetes Care 37:1401–1409
34. Friedman JM, Halaas JL (1998) Leptin and the regulation of body weight in mammals. Nature 95:763–770
35. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF (1996) Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 334:292–295
36. Chen J, Pamuklar Z, Spagnoli A, Torquati A (2012) Serum leptin levels are inversely correlated with omental gene expression of adiponectin and markedly decreased after gastric bypass surgery. Surg Endosc 26:1476–1480
37. Damms-Machado A, Mitra S, Schollenberger AE, Kramer KM, Meile T, Königsrainer A, Huson DH, Bischoff SC (2015) Effects of surgical and dietary weight loss therapy for obesity on gut microbiota composition and nutrient absorption. Biomed Res Int. https://doi.org/10.1155/2015/806248,February1
38. Kong LC, Tap J, Aron-Wisnewsky J, Pelloux V, Basdevant A, Bouillot JL, Zucker JD, Doré J, Clément K (2013) Gut microbiota after gastric bypass in human obesity: increased richness and associations of bacterial genera with adipose tissue genes. Am J Clin Nutr 98:16–24
39. Magalhaes I, Pingris K, Poitou C, Bessoles S, Venteclef N, Kiaf B, Beaudoin L, da Silva J, Allatif O, Rossjohn J, Kjer-Nielsen L, McCluskey J, Ledoux S, Genser L, Torcivia A, Soudais C, Lantz O, Boitard C, Aron-Wisnewsky J, Larger E, Clément K, Lehuen A (2015) Mucosal-associated invariant T cell alterations in obese and type 2 diabetic patients. J Clin Invest 125:1752–1762
40. Toubal A, Kiaf B, Beaudoin L, Cagninacci L, Rhimi M, Fruchet B, da Silva J, Corbett AJ, Simoni Y, Lantz O, Rossjohn J, McCluskey J, Lesnik P, Maguin E, Lehuen A (2020) Mucosal-associated invariant T cells promote inflammation and intestinal dysbiosis leading to metabolic dysfunction during obesity. Nat Commun. https://doi.org/10.1038/s41467-020-17307-0,July24
41. Mazmanian SK, Round JL, Kasper DL (2008) A microbial symbiosis factor prevents intestinal inflammatory disease. Nature 453:620–625
42. Round JL, Mazmanian SK (2010) Inducible $Foxp3^+$ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci USA 107:12204–12209
43. Agabiti-Rosei C, Trapletti V, Piantoni S, Airo P, Tincani A, de Ciuceis C, Rossini C, Mittempergher F, Titi A, Portolani N, Caletti S, Coschignano MA, Porteri E, Tiberio GAM, Pileri P, Solaini L, Kumar R, Ministrini S, Rosei EA, Rizzoni D (2018) Decreased circulating T regulatory lymphocytes in obese patients undergoing bariatric surgery. PLoS ONE. https://doi.org/10.1371/journal.pone.0197178,May14
44. van der Weerd K, Dik WA, Schrijver B, Schweitzer DH, Langerak AW, Drexhage HA, Kiewiet RM, van Aken MO, van Huisstede A, van Dongen JJM, van der Lelij AJ, Staal FJT, van Hagen PM (2012) Morbidly obese human subjects have increased peripheral blood $CD4^+$ T cells with skewing toward a Treg- and Th2-dominated phenotype. Diabetes 61:401–408