

Brief Communication



Downregulation of IL-18 Expression in the Gut by Metformin-induced Gut Microbiota Modulation

Heetae Lee ¹, Jiyeon Kim¹, Jinho An¹, Sungwon Lee¹, Dohyun Choi¹, Hyunseok Kong¹, Youngcheon Song ¹, Il Ho Park¹, Chong-Kil Lee ², Kyungjae Kim ^{1,*}

¹College of Pharmacy, Sahmyook University, Seoul 01795, Korea

²College of Pharmacy, Chungbuk National University, Cheongju 28644, Korea

OPEN ACCESS

Received: Apr 1, 2019

Revised: Aug 7, 2019

Accepted: Aug 14, 2019

*Correspondence to

Kyungjae Kim

College of Pharmacy, Sahmyook University, 815 Hwarang-ro, Nowon-gu, Seoul 01795, Korea.
E-mail: kimkj@syu.ac.kr

Copyright © 2019. The Korean Association of Immunologists

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORCID iDs

Heetae Lee

<https://orcid.org/0000-0001-5105-8841>

Youngcheon Song

<https://orcid.org/0000-0002-4350-6330>

Chong-Kil Lee

<https://orcid.org/0000-0001-9070-341X>

Kyungjae Kim

<https://orcid.org/0000-0002-0943-9306>

Conflicts of Interest

The authors declare no potential conflict of interest.

Abbreviations

FMT, fecal microbiota transplantation; IBD, inflammatory bowel disease; IPGTT, intraperitoneal glucose tolerance testing; HFD, high-fat diet; RD, regular diet; SCFA, short-chain fatty acid; T2D, type 2 diabetes

ABSTRACT

IL-18 is a crucial pro-inflammatory cytokine that mediates chronic intestinal inflammation. Metformin, an anti-diabetic drug, was reported to have ameliorative effects on inflammatory bowel disease. Recently, the mechanism of action of metformin was explained as a modulation of gut microbiota. In this study, fecal microbiota transplantation (FMT) using fecal material from metformin-treated mice was found to upregulate the expression of GLP-1 and pattern-recognition receptors TLR1 and TLR4 for the improvement in hyperglycemia caused by a high-fat diet. Further, FMT downregulated the expression of the inflammatory cytokine IL-18. Within the genera *Akkermansia*, *Bacteroides*, and *Butyrivimonas*, which were promoted by metformin therapy, *Butyrivimonas* was found to be consistently abundant following FMT. Our findings suggest that modulation of gut microbiota is a key factor for the anti-inflammatory effects of metformin which is used for the treatment of hyperglycemia.

Keywords: IL-18; Metformin; Gut microbiota; Fecal microbiota transplantation; Toll-like receptors; GLP-1

INTRODUCTION

Inflammatory bowel disease (IBD) is characterized by chronic inflammation of the gastrointestinal tract. Various immunological factors play an important role in this group of chronic inflammatory disorders. IL-18 is a primary mediator of IBD (1,2). A balance of Th17 cell differentiation and Treg function is crucial for chronic intestinal inflammation, which is reported to be regulated by IL-18.

Metformin is a first-line medication used for the treatment of type 2 diabetes (T2D) and is widely prescribed in patients with hyperglycemia and obesity-related metabolic syndrome. Metformin treatment has been reported to have various beneficial effects, such as anti-inflammatory and anti-cancer effects (3,4). Recent studies have demonstrated that metformin ameliorates the symptoms of IBD (5).

Gut microbiota is an important environmental factor that affects energy metabolism and immune homeostasis (6-8). In recent studies, modulation of the gut microbiota by metformin

Author Contributions

Conceptualization: Lee H; Data curation: Lee H, Kim J; Formal analysis: Lee H, Kim J, An J, Choi D; Investigation: Lee H, Kim J, An J; Project administration: Kong H, Song Y, Kim K; Supervision: Kim K; Writing - original draft: Lee H; Writing - review & editing: Kim K, Lee CK, Park IH.

therapy led to a consistent metabolic improvement with a significant increase in the abundance of *Akkermansia muciniphila* (9-12). Moreover, a significant increase in butyrate-producing bacteria, such as *Bacteroides* and *Butyricimonas* species, was observed in an aged mouse model (13).

Fecal microbiota transplantation (FMT) is widely used to confirm the causative effects of modulated gut microbiota by certain interventions. In this study, we aimed to evaluate the effects of allogeneic FMT on metabolic parameters by a transplant of fecal material from metformin-treated mice to antibiotic-treated mice, and investigate immunological changes following FMT to clarify the anti-inflammatory effects of metformin via gut microbiota modulation.

MATERIALS AND METHODS

FMT

Fecal material was collected from a metformin-treated mice model as described in a previous study (13). Briefly, 6-week-old male C57BL/6N mice were fed a high-fat diet (HFD) (45% kcal fat; FeedLab Inc., Hanam, Korea) for 39 weeks to induce metabolic disorders. Metformin (250 mg/kg) was administered daily for the final 16 weeks of HFD feeding (HFD-Met mice group). Prior to FMT, the mice were provided drinking water containing antibiotics (penicillin G procaine [2,000 IU/L] and streptomycin [2.5 mg/L]) for 5 days. Fecal material (0.1 g) from the metformin-treated mice was pooled in 1 mL of PBS. After centrifugation at 2,000×g for 2 min, 500 µL supernatant was administered using an oral gavage to the antibiotic-treated mice that were fed with an HFD for 48 wk (HFD-fMet, n=6). All experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Sahmyook University and were conducted in accordance with the Guide for the Care and Use of Laboratory Animal (SYUIACUC 2015001).

Metabolic parameters

Body weight, serum glucose level, and food intake were recorded once every second week. Serum glucose level was estimated using an Accu-Chek Performa system (Roche Diagnostics, Mannheim, Germany) following fasting for 12 h. Intraperitoneal glucose tolerance testing (IPGTT) was performed at 16 wk after metformin administration. The mice were intraperitoneally injected with glucose solution (2 g/kg in PBS), and the glucose level was estimated 30, 60, 90, and 120 min after injection. The total cholesterol was estimated using a biochemical analyzer (AU480; Beckman Coulter, Brea, CA, USA).

Gut microbiota analysis

Total DNA was extracted using a PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) from cecum samples including fecal material according to the manufacturer's instructions. Based on a previous study, the relative abundance of 3 bacterial genera (*Akkermansia*, *Bacteroides*, and *Butyricimonas*) in the cecum was confirmed using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and a StepOnePlus real-time PCR system (Applied Biosystems). The genus-specific primer sets for target bacterial 16S rDNA were described in previous study (13).

Transcriptome analysis

The expression of IL-1β (forward primer: 5'-CAGGATGAGGACATGACACC-3', reverse primer: 5'-CTCTGCAGACTCAAACCTCCAC-3'), IL-6 (forward primer: 5'-GTACTCCAGAAGACCAGAGC-3', reverse primer: 5'-TGC TGG TGA CAA CCA CGG CC-3'), TGF-β1 (forward primer:

5'-GCGGACTACTATGCTAAAGAGG-3', reverse primer: 5'-GTAGAGTTCACATGTTGCTCC-3'), IL-4 (forward primer: 5'-GAGCCATATCCACGGATGCGACAA-3', reverse primer: 5'-CATGGTGGCTCAGTACTACGAGTA-3'), TNF- α (forward primer: 5'-GCCTCTTCTCATTCTGCTTG-3', reverse primer: 5'-CTGATGAGAGGGAGGCCATT-3'), NF- κ B (forward primer: 5'-GAAATTCCTGATCCAGACAAAAAC-3', reverse primer: 5'-ATCACTTCAATGGCCTCTGTGTAG-3'), and IL-18 (forward primer: 5'-CAGGCCTGACATCTTCTGCAA-3', reverse primer: 5'-TCTGACATGGCAGCCATTGT-3'), TLR1 (forward primer: 5'-CAATGTGGAAACAACGTGGA-3', reverse primer: 5'-TGTAACCTTGGGGGAAGCTG-3'), TLR2 (forward primer: 5'-AAGAGGAAGCCCAAGAAAGC-3', reverse primer: 5'-CGATGGAATCGATGATGTTG-3'), TLR4 (forward primer: 5'-ACCTGGCTGGTTTACACGTC-3', reverse primer: 5'-CTGCCAGAGACATGCAGAA-3'), TLR5 (forward primer: 5'-AAGTTCGGGGAATCTGTTT-3', reverse primer: 5'-GCATAGCCTGAGCCTGTTTC-3'), TLR6 (forward primer: 5'-TTCCCAATACCACCGTTCTC-3', reverse primer: 5'-CTATGTGCTGGAGGGTCACA-3'), GLP-1 (forward primer: 5'-GGGTCTCTGGCTACATAAGGACAAC-3', reverse primer: 5'-AAGGATGGCTGAAGCGATGAC-3'), DPP4 (forward primer: 5'-AAATGGGATTTGTGGACAGCAAG-3', reverse primer: 5'-CCGATCCCAGGACCATTGAG-3'), and GAPDH (forward primer: 5'-AACTTTGGCATTGTGGAAGG-3', reverse primer: 5'-ACACATTGGGGTAGGAACA-3') were determined in the ileum including the Peyer's patches. Total RNA was extracted using a RiboEx (GeneAll, Seoul, Korea) and cDNA synthesis was performed using a HyperScript RT premix (GeneAll) according to the manufacturer's instructions. SYBR Green PCR Master Mix (Applied Biosystems) and a StepOnePlus real-time PCR system (Applied Biosystems) was used to quantify the mRNA levels. GAPDH was used as an internal control. The primer sets for transcriptome were described in previous study (13).

Western blot analysis

Ileum tissue were homogenized in RIPA cell lysis buffer (GenDEPOT, Katy, TX, USA) added with protease inhibitor cocktail solution (GenDEPOT). To perform the western blot analysis of IL-18, protein lysates (80 μ g) were separated by electrophoresis. The blots were blocked for 1–2 h with 5% BSA in TBST solution (Tris buffered saline containing 0.05% Tween-20), and incubated overnight at 4°C with primary antibodies: anti-GAPDH and anti-IL-18 polyclonal antibodies (Abcam, Cambridge, UK). After repeated washings, anti-rabbit IgG HRP-conjugated antibody (GenDEPOT) were added for 1 h, immunoreactive bands were visualized using a chemiluminescent peroxidase substrate (ECL Plus; GenDEPOT) and image system (Chemidoc XRS system; Bio-Rad, Hercules, CA, USA).

Statistical analysis

All data are presented as the means \pm SEM. The $2^{-\Delta\Delta C_t}$ relative quantification method [$\Delta\Delta C_t = (C_{t,Target} - C_{t,\beta\text{-actin}})_{Group 1} - (C_{t,Target} - C_{t,\beta\text{-actin}})_{Group 2}$] was used to quantify the *in vivo* mRNA levels relative to the internal control (GAPDH). The statistical significance was assessed by one-way ANOVA followed by Duncan's *post hoc* test. All statistical analyses were performed using R Studio software (RStudio, Inc., Boston, MA, USA). The statistical significance was determined at $p < 0.05$.

RESULTS

Effects of FMT on metabolic parameters and the gut microbiota

Serum glucose level (154.2 \pm 8.4 mg/dL) and IPGTT (991.5 \pm 184.8) increased more after FMT involving fecal materials from the HFD-Met mice group than from the HFD group (192.0 \pm 27.6

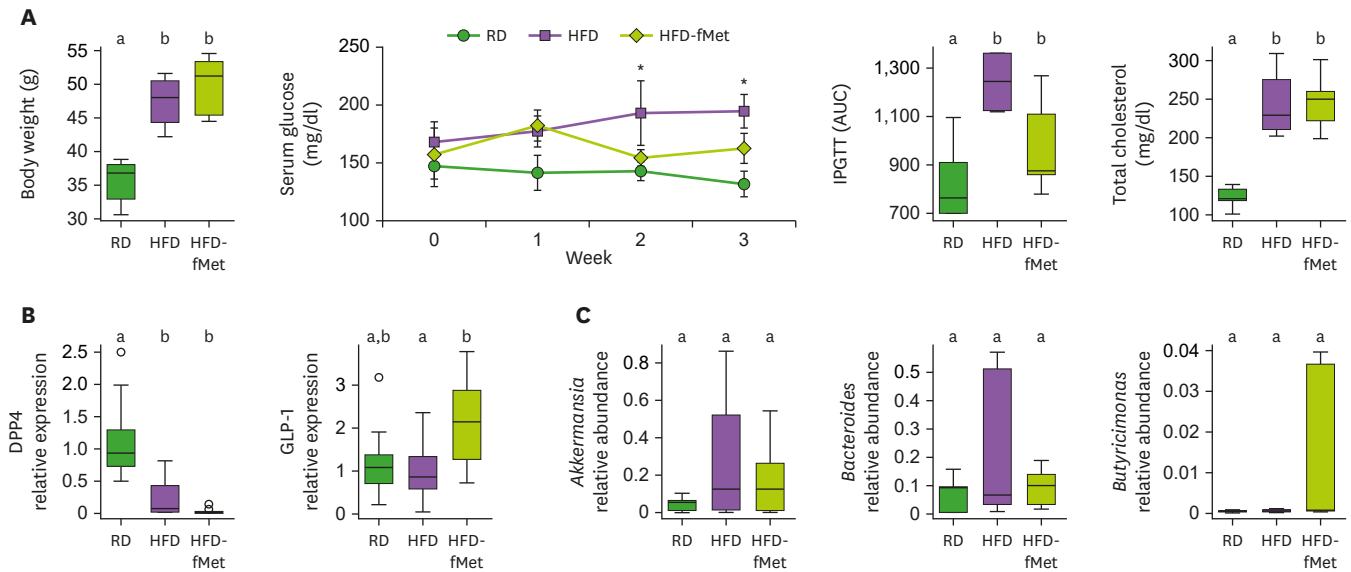


Figure 1. Changes in the metabolic profiles and gut microbiota after FMT (A) metabolic profiles are shown. Six-week-old mice were fed on HFD for 43 wk after which pooled fecal material from metformin-treated mice (HFD-fMet, n=6) was orally transferred once to the antibiotic-treated mice. Body weight, IPGTT, and total cholesterol were measured 4 wk after FMT [RD (n=5), HFD (n=4)]. The serum glucose level was measured after fasting for 12 h. (B) Relative mRNA level of DPP4 and GLP-1 as determined by quantitative PCR in the ileum. (C) The relative abundance of bacterial genera determined by quantitative PCR in the cecum. Different superscript letters indicate significant differences ($p < 0.05$) according to Duncan's *post hoc* test. *Statistical significance between HFD and HFD-fMet groups ($p < 0.05$).

mg/dL and $1,243.5 \pm 137.5$, respectively). This effect lasted for 4 weeks, although the increase in IPGTT was not significant (Fig. 1A). Body weight and total cholesterol did not differ significantly between the HFD and HFD-fMet groups. GLP-1 expression was significantly higher in the HFD-fMet group than in the HFD group (Fig. 1B). The relative abundances of *Akkermansia*, *Bacteroides*, and *Butyricimonas* were not statistically significant between the HFD and HFD-fMet groups (Fig. 1C).

Effects of FMT on immune responses in the ileum

TLR1 and TLR4 expression levels were significantly higher in the HFD-fMet group than in the HFD group (Fig. 2A). TLR2, TLR5, and TLR6 did not differ significantly between the HFD and FMT groups. The expression of TLRs were not significantly different between RD and HFD groups. Among the pro-inflammatory cytokines, IL-18 expression was significantly lower in the HFD-fMet group than in the HFD group; this was the only significant difference (Fig. 2B). In addition, relative protein expression level of IL-18/GAPDH was 1.24 ± 0.11 , 1.37 ± 0.72 , and 0.95 ± 0.28 in RD, HFD, and HFD-fMet group, respectively (Fig. 2C).

DISCUSSION

Recently, modulation of the gut microbiota by metformin treatment has been shown to be a causative factor for metabolic improvement, such that FMT from metformin-treated donors improved the glucose tolerance of germ-free mice (10,13). As anti-inflammatory effect of metformin was reported as well, which is expected to have a relation with the modulation of the pre-existing gut microbiota by metformin treatment.

Interestingly, gut microbiota modulation by metformin treatment was related to an increase in the short-chain fatty acid (SCFA)-producing bacteria (13), *Akkermansia*, *Butyricimonas*, and

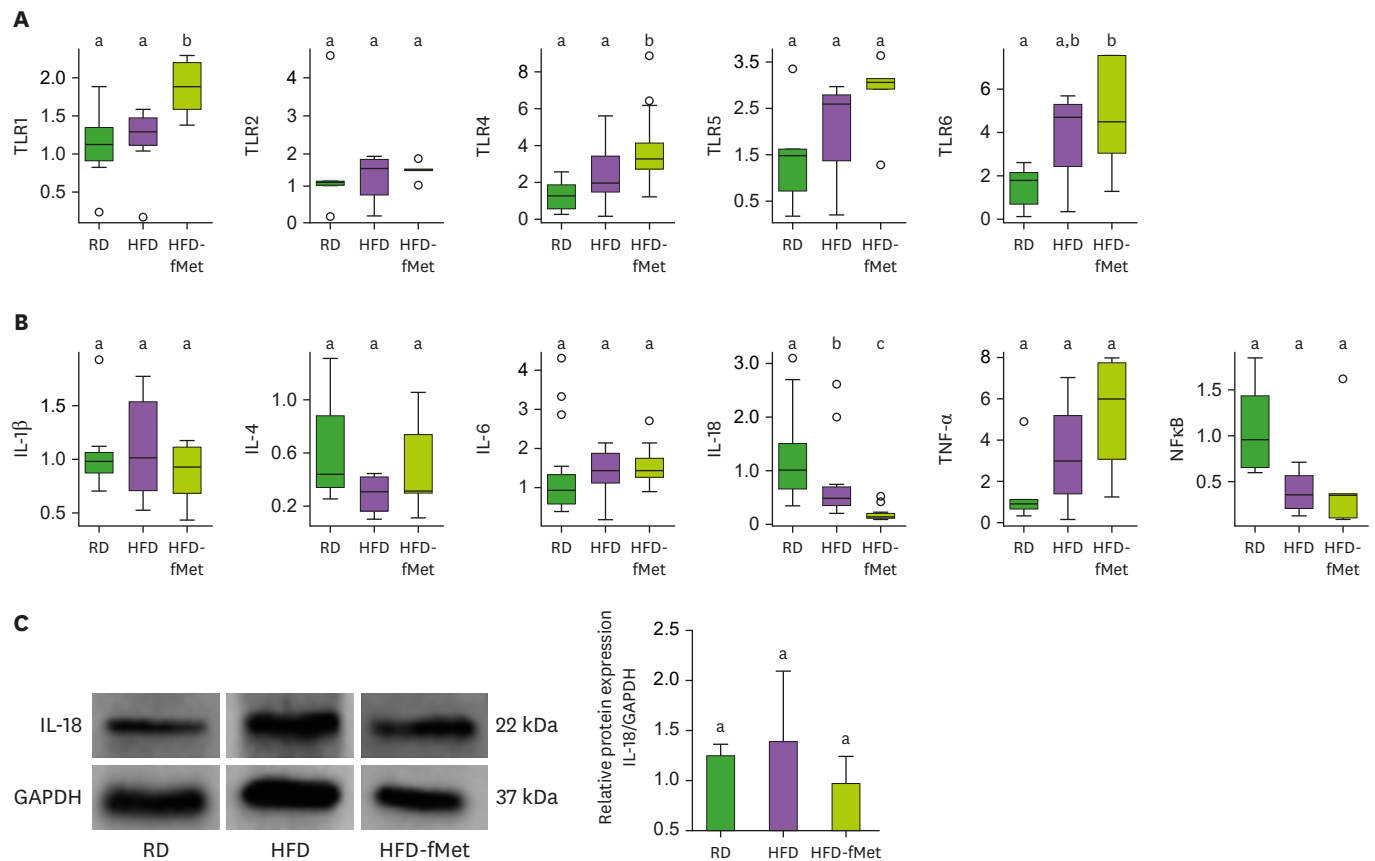


Figure 2. Immunological changes in the ileum after FMT. (A) Relative mRNA levels of TLRs. (B) Relative mRNA levels of the inflammatory cytokines. (C) Western blot analysis of IL-18 in the ileum. Different superscript letters indicate significant differences ($p < 0.05$) according to Duncan's *post hoc* test.

Bacteroides. Akkermansia (*A. muciniphila*), considered to be a next-generation probiotic, is a mucin-degrading species that produces propionate, the abundance of which is associated with anti-inflammatory effects, antioxidant effects, and metabolic improvements (14-16). Moreover, the abundance of *Akkermansia* has been reported to be significantly higher in patients with T2D treated with metformin and was found to be increased by metformin treatment in an animal model regardless of the age (9,11,13). *Bacteroides* is an SCFA-producing bacterium that is abundant in non-obese individuals (17). Several strains of *Bacteroides* produce propionate and succinate, which protect against various obesity-related metabolic disorders, such as insulin resistance (18). Recently, the dipeptidyl peptidase-4 inhibitor vildagliptin has been shown to decrease the abundance of butyrate-producing bacteria including *Bacteroides* in the intestines of diabetic rats (19). However, after FMT using fecal material from the HFD-Met mouse group, which contained abundant *Akkermansia* and *Bacteroides* populations, only the glucose parameters improved but not body weight or lipid parameters. FMT also increased the abundance of *Butyricimonas*, suggesting that *Butyricimonas* plays an important role in the regulation of hyperglycemia after FMT. The increased abundance of *Butyricimonas* was associated with improved metabolic parameters in the aged and adult mice treated with metformin (9). Butyrate, which is produced by *Butyricimonas* spp., protects against diet-induced obesity (20). Moreover, butyrate increased the mucosal layer thickness in the intestine. This parameter is also regulated by *Akkermansia*, which has been associated with metabolic improvements, including glucose tolerance (21,22). On the other hands, pro-inflammatory cytokines were induced by SCFAs in a time- and dose-dependent

manner (23). Pro-inflammatory roles of activated free fatty acid receptors, involved in inflammation and immune responses by SCFAs, were also reported (24). Therefore, the adequate amount of SCFAs should be considered for beneficial effects on anti-inflammation and metabolic improvements.

TLR expression patterns provide insights into the mechanism of FMT in glucose regulation. During the treatment of *Clostridium difficile* infection, FMT modulates the tight junctions and mucus structure and stimulates the mucosal immune system in the intestine (25). However, the mechanism of FMT in metabolic improvements has remained unclear. To clarify this mechanism, we investigated the regulation of TLRs that recognizes different molecular patterns of the microorganism. TLR1 recognizes bacterial lipoproteins and peptidoglycans and is known to mediate cytokine production along with TLR2. Recently, TLR1 has been reported to play an important role in the regulation of chronic inflammation by sensing the gut microbiota (26). In another study, TLR1 signaling was shown to regulate inflammation during intestinal infection, and TLR1 deficiency led to vulnerability to intestinal inflammation and tissue injury by dysbiosis (27). Moreover, drug efficacy has been shown to be affected by gut microbiota, such that TLR1 was upregulated in healthy individuals with good glycemic control without any complication (28). TLR4 recognizes lipopolysaccharides derived from bacteria, insulin resistance, and inflammation by TLR4 signaling mediated by NF- κ B (29). Dysbiosis contributes to the development of colonic inflammation and metabolic disorders including T2D via TLR4 signaling (30). However, in the current study, TLR4 expression was increased by FMT, and NF- κ B remained unchanged, although the regulatory effects of TLR4 on hyperglycemia not mediated by NF- κ B was considered. Recently, TLR4 was reported to play an important role as a recognition receptor controlling the sensitivity of GLP-1, which is a therapeutic target for T2D (31). Therefore, mediation of gut microbiota modulation by TLR1 and TLR4 signaling may be associated with hyperglycemia regulation, although the specific roles of TLR1 and TLR4 remain unknown.

GLP-1 upregulation may play a key role in glucose regulation after FMT therapy. GLP-1 is an insulinotropic hormone, whereas dysbiosis is responsible for GLP-1 resistance (31). Therefore, glucose regulation after FMT was caused by the therapeutic modulation of intestinal dysbiosis by metformin. Although activation of AMP-activated protein kinase is a well-known mechanism of action of metformin, it also induces GLP-1 secretion, which may be associated with modulation of gut microbiota. Interestingly, GLP-1 was increased in chronic inflammatory states and related to immune responses in the gut (32). In addition to the anti-hyperglycemic effects, GLP-1-based therapies have shown anti-inflammatory effects in chronic inflammatory disease, such as T2D and atherosclerosis (33,34).

Metformin has beneficial effects on glucose regulation and causes improvement in the symptoms of IBD (5), which may be associated with modulation of the gut microbiota. IL-18 is an IL-1 family inflammatory cytokine that contributes to IBD pathology and acts as an indicator of IBD progression (35). IL-18/IL-18R signaling in the intestinal epithelial cells plays a pivotal role in the progression of IBD. The regulation of CD4⁺ T cells and Foxp3⁺ Treg cells are modulated during intestinal inflammation (1,36). Therefore, neutralization of IL-18 activity was suggested to ameliorate intestinal inflammation (37). Metformin therapy was reported to inhibit IL-18 secretion in patients with T2D, and interestingly, consistent results were observed following FMT treatment in the current study (38). Additionally, recently reported anti-cancer and anti-aging effects of metformin might have been mediated by the modulation of the gut microbiota (3).

In summary, based on recent results that metformin improves metabolic disorders including obesity, hyperglycemia, and hyperlipidemia via gut microbiota modulation, we determined that FMT using fecal material from metformin-treated mice modulated the anti-inflammatory immune responses as well as glucose regulation. The mechanism underlying the effects of FMT on metabolic improvements is unclear; however, the use of FMT by upregulation of GLP-1 may play a key role in glucose regulation mediated by TLR1 and TLR4 after FMT. Above all, the anti-inflammatory effects of metformin may be associated with inhibition of IL-18 via gut microbiota modulation.

ACKNOWLEDGEMENTS

This study was supported by the Sahmyook University Research Fund (RI12019069 [2019]).

REFERENCES

1. Harrison OJ, Srinivasan N, Pott J, Schiering C, Krausgruber T, Ilott NE, Maloy KJ. Epithelial-derived IL-18 regulates Th17 cell differentiation and Foxp3⁺ Treg cell function in the intestine. *Mucosal Immunol* 2015;8:1226-1236.
[PUBMED](#) | [CROSSREF](#)
2. Guan Q, Zhang J. Recent advances: the imbalance of cytokines in the pathogenesis of inflammatory bowel disease. *Mediators Inflamm* 2017;2017:4810258.
[PUBMED](#) | [CROSSREF](#)
3. Berstein LM. Metformin in obesity, cancer and aging: addressing controversies. *Aging (Albany NY)* 2012;4:320-329.
[PUBMED](#) | [CROSSREF](#)
4. Cameron AR, Morrison VL, Levin D, Mohan M, Forteach C, Beall C, McNeilly AD, Balfour DJ, Savinko T, Wong AK, et al. Anti-inflammatory effects of metformin irrespective of diabetes status. *Circ Res* 2016;119:652-665.
[PUBMED](#) | [CROSSREF](#)
5. Lee SY, Lee SH, Yang EJ, Kim EK, Kim JK, Shin DY, Cho ML. Metformin ameliorates inflammatory bowel disease by suppression of the STAT3 signaling pathway and regulation of the between Th17/Treg balance. *PLoS One* 2015;10:e0135858.
[PUBMED](#) | [CROSSREF](#)
6. Sanz Y, Olivares M, Moya-Pérez Á, Agostoni C. Understanding the role of gut microbiome in metabolic disease risk. *Pediatr Res* 2015;77:236-244.
[PUBMED](#) | [CROSSREF](#)
7. Wu HJ, Wu E. The role of gut microbiota in immune homeostasis and autoimmunity. *Gut Microbes* 2012;3:4-14.
[PUBMED](#) | [CROSSREF](#)
8. Nagao-Kitamoto H, Kamada N. Host-microbial cross-talk in inflammatory bowel disease. *Immune Netw* 2017;17:1-12.
[PUBMED](#) | [CROSSREF](#)
9. Lee H, Ko G. Effect of metformin on metabolic improvement and gut microbiota. *Appl Environ Microbiol* 2014;80:5935-5943.
[PUBMED](#) | [CROSSREF](#)
10. Wu H, Esteve E, Tremaroli V, Khan MT, Caesar R, Mannerås-Holm L, Ståhlman M, Olsson LM, Serino M, Planas-Félix M, et al. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nat Med* 2017;23:850-858.
[PUBMED](#) | [CROSSREF](#)
11. Mardinoglu A, Boren J, Smith U. Confounding effects of metformin on the human gut microbiome in type 2 diabetes. *Cell Metab* 2016;23:10-12.
[PUBMED](#) | [CROSSREF](#)
12. Shin NR, Lee JC, Lee HY, Kim MS, Whon TW, Lee MS, Bae JW. An increase in the *Akkermansia* spp. Population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. *Gut* 2014;63:727-735.
[PUBMED](#) | [CROSSREF](#)

13. Lee H, Lee Y, Kim J, An J, Lee S, Kong H, Song Y, Lee CK, Kim K. Modulation of the gut microbiota by metformin improves metabolic profiles in aged obese mice. *Gut Microbes* 2018;9:155-165.
[PUBMED](#) | [CROSSREF](#)
14. Zhao S, Liu W, Wang J, Shi J, Sun Y, Wang W, Ning G, Liu R, Hong J. *Akkermansia muciniphila* improves metabolic profiles by reducing inflammation in chow diet-fed mice. *J Mol Endocrinol* 2017;58:1-14.
[PUBMED](#) | [CROSSREF](#)
15. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, Guiot Y, Derrien M, Muccioli GG, Delzenne NM, et al. Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci U S A* 2013;110:9066-9071.
[PUBMED](#) | [CROSSREF](#)
16. Schneeberger M, Everard A, Gómez-Valadés AG, Matamoros S, Ramírez S, Delzenne NM, Gomis R, Claret M, Cani PD. *Akkermansia muciniphila* inversely correlates with the onset of inflammation, altered adipose tissue metabolism and metabolic disorders during obesity in mice. *Sci Rep* 2015;5:16643.
[PUBMED](#) | [CROSSREF](#)
17. Kasai C, Sugimoto K, Moritani I, Tanaka J, Oya Y, Inoue H, Tameda M, Shiraki K, Ito M, Takei Y, et al. Comparison of the gut microbiota composition between obese and non-obese individuals in a Japanese population, as analyzed by terminal restriction fragment length polymorphism and next-generation sequencing. *BMC Gastroenterol* 2015;15:100.
[PUBMED](#) | [CROSSREF](#)
18. Rios-Covian D, Salazar N, Gueimonde M, de Los Reyes-Gavilan CG. Shaping the metabolism of intestinal bacteroides population through diet to improve human health. *Front Microbiol* 2017;8:376.
[PUBMED](#) | [CROSSREF](#)
19. Zhang Q, Xiao X, Li M, Yu M, Ping F, Zheng J, Wang T, Wang X. Vildagliptin increases butyrate-producing bacteria in the gut of diabetic rats. *PLoS One* 2017;12:e0184735.
[PUBMED](#) | [CROSSREF](#)
20. Lin HV, Frassetto A, Kowalik EJ Jr, Nawrocki AR, Lu MM, Kosinski JR, Hubert JA, Szeto D, Yao X, Forrest G, et al. Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. *PLoS One* 2012;7:e35240.
[PUBMED](#) | [CROSSREF](#)
21. Cornick S, Tawiah A, Chadee K. Roles and regulation of the mucus barrier in the gut. *Tissue Barriers* 2015;3:e982426.
[PUBMED](#) | [CROSSREF](#)
22. Carvalho BM, Saad MJ. Influence of gut microbiota on subclinical inflammation and insulin resistance. *Mediators Inflamm* 2013;2013:986734.
[PUBMED](#) | [CROSSREF](#)
23. Mirmonsef P, Zariwaf MR, Gilbert D, Makinde H, Landay AL, Spear GT. Short-chain fatty acids induce pro-inflammatory cytokine production alone and in combination with toll-like receptor ligands. *Am J Reprod Immunol* 2012;67:391-400.
[PUBMED](#) | [CROSSREF](#)
24. Li M, van Esch BC, Wagenaar GT, Garssen J, Folkerts G, Henricks PA. Pro- and anti-inflammatory effects of short chain fatty acids on immune and endothelial cells. *Eur J Pharmacol* 2018;831:52-59.
[PUBMED](#) | [CROSSREF](#)
25. Khoruts A, Sadowsky MJ. Understanding the mechanisms of faecal microbiota transplantation. *Nat Rev Gastroenterol Hepatol* 2016;13:508-516.
[PUBMED](#) | [CROSSREF](#)
26. Kamdar K, Johnson AM, Chac D, Myers K, Kulur V, Truevillian K, DePaolo RW. Innate recognition of the microbiota by TLR1 promotes epithelial homeostasis and prevents chronic inflammation. *J Immunol* 2018;201:230-242.
[PUBMED](#) | [CROSSREF](#)
27. Kamdar K, Khakpour S, Chen J, Leone V, Brulc J, Mangatu T, Antonopoulos DA, Chang EB, Kahn SA, Kirschner BS, et al. Genetic and metabolic signals during acute enteric bacterial infection alter the microbiota and drive progression to chronic inflammatory disease. *Cell Host Microbe* 2016;19:21-31.
[PUBMED](#) | [CROSSREF](#)
28. Gupta S, Maratha A, Siednienko J, Natarajan A, Gajanayake T, Hoashi S, Miggin S. Analysis of inflammatory cytokine and TLR expression levels in Type 2 Diabetes with complications. *Sci Rep* 2017;7:7633.
[PUBMED](#) | [CROSSREF](#)
29. Kim F, Pham M, Luttrell I, Bannerman DD, Tupper J, Thaler J, Hawn TR, Raines EW, Schwartz MW. Toll-like receptor-4 mediates vascular inflammation and insulin resistance in diet-induced obesity. *Circ Res* 2007;100:1589-1596.
[PUBMED](#) | [CROSSREF](#)

30. Velloso LA, Folli F, Saad MJ. TLR4 at the crossroads of nutrients, gut microbiota, and metabolic inflammation. *Endocr Rev* 2015;36:245-271.
[PUBMED](#) | [CROSSREF](#)
31. Grasset E, Puel A, Charpentier J, Collet X, Christensen JE, Terce F, Burcelin R. A specific gut microbiota dysbiosis of type 2 diabetic mice induces GLP-1 resistance through an enteric NO-dependent and gut-brain axis mechanism. *Cell Metab* 2017;25:1075-1090.e5.
[PUBMED](#) | [CROSSREF](#)
32. Zietek T, Rath E. Inflammation meets metabolic disease: gut feeling mediated by GLP-1. *Front Immunol* 2016;7:154.
[PUBMED](#) | [CROSSREF](#)
33. Lee YS, Jun HS. Anti-inflammatory effects of GLP-1-based therapies beyond glucose control. *Mediators Inflamm* 2016;2016:3094642.
[PUBMED](#) | [CROSSREF](#)
34. Insuela DB, Carvalho VF. Glucagon and glucagon-like peptide-1 as novel anti-inflammatory and immunomodulatory compounds. *Eur J Pharmacol* 2017;812:64-72.
[PUBMED](#) | [CROSSREF](#)
35. Kanai T, Kamada N, Hisamatsu T. Clinical strategies for the blockade of IL-18 in inflammatory bowel diseases. *Curr Drug Targets* 2013;14:1392-1399.
[PUBMED](#) | [CROSSREF](#)
36. Nowarski R, Jackson R, Gagliani N, de Zoete MR, Palm NW, Bailis W, Low JS, Harman CC, Graham M, Elinav E, et al. Epithelial IL-18 equilibrium controls barrier function in colitis. *Cell* 2015;163:1444-1456.
[PUBMED](#) | [CROSSREF](#)
37. Sivakumar PV, Westrich GM, Kanaly S, Garka K, Born TL, Derry JM, Viney JL. Interleukin 18 is a primary mediator of the inflammation associated with dextran sulphate sodium induced colitis: blocking interleukin 18 attenuates intestinal damage. *Gut* 2002;50:812-820.
[PUBMED](#) | [CROSSREF](#)
38. Lee HM, Kim JJ, Kim HJ, Shong M, Ku BJ, Jo EK. Upregulated NLRP3 inflammasome activation in patients with type 2 diabetes. *Diabetes* 2013;62:194-204.
[PUBMED](#) | [CROSSREF](#)