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Original Research

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Effect of vegetable oils with different fatty acid composition on high-fat diet-induced obesity and colon inflammation

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ABSTRACT

BACKGROUND/OBJECTIVES: Different fatty acids exert different health benefits. This study investigated the potential protective effects of perilla, olive, and safflower oils on high-fat diet-induced obesity and colon inflammation.
MATERIALS/METHODS: Five-week old, C57BL/6J mice were assigned to 5 groups: low-fat diet (LFD), high-fat diet (HFD) and high-fat diet supplemented with-perilla oil (HPO), olive oil (HOO), and safflower oil (HSO). After 16 weeks of the experimental period, the mice were sacrificed, and blood and tissues were collected. The serum was analyzed for obesity- and inflammation-related biomarkers. Gene expression of the biomarkers in the liver, adipose tissue, and colon tissue was analyzed. Micro-computed tomography (CT) analysis was performed one week before sacrifice.
RESULTS: Treatment with all the three oils significantly improved obesity-induced increases in body weight, liver weight, and epididymal fat weight as well as serum triglyceride and leptin levels. Treatment with perilla oil (PO) and safflower oil (SO) increased adiponectin levels. The micro-CT analysis revealed that PO and SO reduced abdominal fat volume considerably. The mRNA expression of lipogenic genes was reduced in all the three oil-supplemented groups and PO upregulated lipid oxidation in the liver. Supplementation of oils improved macroscopic score, increased colon length, and decreased serum endotoxin and proinflammatory cytokine levels in the colon. The abundance of *Bifidobacteria* was increased and that of *Enterobacteriaceae* was reduced in the PO-supplemented group. All three oils reduced proinflammatory cytokine levels, as indicated by the mRNA expression. In addition, PO increased the expression of tight junction proteins.
CONCLUSIONS: Taken together, our data indicate that the three oils exert similar anti-obesity effects. Interestingly, compared with olive oil and SO, PO provides better protection against high-fat diet-induced colon inflammation, suggesting that PO consumption helps manage inflammation-related diseases and provides omega-3 fatty acids needed by the body.

Keywords: Perilla; olive oil; safflower oil; inflammation

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Conflict of Interest
The authors declare no potential conflicts of interests.

Author Contributions
Conceptualization: Thomas SS, Cha YS, Kim KA; Formal analysis: Thomas SS, Kim KA; Funding acquisition: Kim KA; Investigation: Thomas SS, Kim KA; Methodology: Thomas SS, Kim KA; Supervision: Cha YS, Kim KA; Writing - original draft: Thomas SS; Writing - review & editing: Cha YS, Kim KA.

INTRODUCTION

Since the past few decades, obesity and its related complications have globally been a topic of great interest. Despite many measures being taken, the prevalence of obesity, mainly in developing countries, continues to increase; it is estimated that by 2025, 18% of men and 21% of women worldwide will be obese [1]. Fat overload in the adipose tissue leads to adipocyte dysfunction, which may stimulate the development of different metabolic complications by affecting glucose and lipid metabolism and increasing inflammatory responses [2]. Chronic conditions such as diabetes, hypertension, cardiovascular diseases, and cancer are associated with obesity, contributing to increases in morbidity and mortality rates and economic burden [3]. The concept of meta-inflammation was introduced to explain the chronic low-grade inflammatory response, which is considered crucial in stimulating obesity-related complications. A characteristic feature of obesity-associated meta-inflammation is that it causes chronic low-grade activation of the innate immune system, eventually affecting metabolic homeostasis [4]. Adipose tissue is a major site of inflammatory responses initiated by macrophage infiltration into the tissue and proinflammatory cytokine production. Activation of the inflammatory nuclear factor kappa B (NF-κB) pathway by endotoxins such as lipopolysaccharide (LPS) via membrane receptor Toll-like receptor 4 (TLR4) in fat cells is among the suggested mechanisms that trigger obesity-associated inflammation [5]. Long-term high-fat diet (HFD) consumption causes a microbial imbalance in the gut, creating suitable conditions for the growth of gram-negative bacteria that produce LPS. This LPS can translocate into the systemic circulation and initiate inflammatory responses. In addition, dietary fat affects intestinal permeability by affecting intestinal tight junction proteins, allowing more LPS to translocate into the blood [6]. The integrity of the gut barrier, which act as a shield protecting the body from various pathogens and toxins, should be maintained so that the intestine functions normally. Changes in dietary pattern and sedentary lifestyle are the major factors contributing to obesity. Hence, including foods with health benefits in our daily diet is the most effective way to maintain health.

Oils are an inevitable component of every cuisine, and oils used in different regions have different fatty acid compositions based on their source, availability, and the processing methods used [7]. Perilla oil (PO) is one of the major oils in Korean cuisine, and it is a rich source of n-3 polyunsaturated fatty acids (PUFAs), particularly alpha-linolenic acid (ALA). ALA acts as the precursor for the synthesis of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are omega-3-fatty acids containing several health benefits [8]. Olive oil (OO), an inevitable component of the Mediterranean diet, is rich in oleic acid, which is a monounsaturated fatty acid (MUFA) and is known to reduce the risk of cardiovascular disease [9]. Safflower oil (SO) is another widely used cooking oil. Some reports have shown that SO diet reduce fat mass compared to high-butter diet and increase diet-induced thermogenesis compared to a lard diet [10].

Over the past decade, many studies have shown that the type of fatty acid consumed in high concentration, the imbalance between saturated fatty acid (SFA) and unsaturated fatty acids (USFA), and the ratio of omega-6/omega-3 fatty acids can affect susceptibility towards certain diseases [11,12]. Though various types of oils are used in our daily life, studies comparing the health effects of oils with different fatty acid compositions are few [13,14]. Therefore, based on some previous studies that have reported that PO, OO, and SO have lipid-lowering effects, this study aimed to investigate the effects of these three vegetable oils on HFD-induced obesity and colon inflammation.

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MATERIALS AND METHODS

Diet and animals

Commercially available PO, OO, and SO were purchased from a local supermarket. Five-week-old C57BL/6J mice were obtained from Chung-ang Bio (Seoul, Korea). The mice were divided into five groups after one week of acclimatization: LFD (low-fat diet), HFD (high-fat diet), HPO (high-fat diet supplemented with PO), HOO (high-fat diet supplemented with OO), and HSO (high-fat diet supplemented with SO). The major fatty acids present in the oils used in the study were as follows; PO (ALA-59.5%, oleic acid-14.2%, linoleic acid-12.3%, palmitic acid-5.8%), OO (oleic acid-71.2%, palmitic acid-11.51%, linoleic acid-6.15%), and SO (oleic acid-72.4%, linoleic acid-13.6%, palmitic acid-4.7%). The fatty acid composition of the oils used in the study is given in **Supplementary Table 1**. The experimental diet was prepared weekly. The composition of the diet is presented in **Table 1**. As this study mainly aimed to investigate the effect of the three vegetable oils which contains unsaturated fatty acids as its major component, the experimental diet was made such that 25% (w/w) of lard in 60% kcal% fat diet (245 g lard in 270 g total fats; **Table 1**) was replaced with respective oils and the total amount of oil in the diet was 8 g/100 g diet (weight/weight).

Body weight was measured weekly and diet intake every other day during the 16-week experimental period. The mice were maintained in a housing of 23 ± 1°C, with alternate 12 h light and dark cycles. All the experiments were carried out following the guidelines of the Animal Care and Use Committee of Chungnam National University (CNU-00918).

Micro-computed tomography (micro-CT) analysis for abdominal fat volume

The abdominal fat volume of mice (n=3) was analyzed using micro-CT analysis, one week before sacrifice. Briefly, the mice were anesthetized with Rompun: Zoletil 50 in the ratio 1:1. The

Table 1. Components of the experimental diet fed to the mice

Ingredient (g)	LFD	HFD	HPO	HOO	HSO
Casein	200	200	200	200	200
L-cysteine	3	3	3	3	3
Corn starch	506.2	0	0	0	0
Maltodextrin	125	125	125	125	125
Sucrose	68.8	68.8	68.8	68.8	68.8
Cellulose	50	50	50	50	50
Soybean oil	25	25	25	25	25
Lard	20	245	182	182	182
Dicalcium phosphate	13	13	13	13	13
Calcium carbonate	5.5	5.5	5.5	5.5	5.5
Potassium citrate	16.5	16.5	16.5	16.5	16.5
Mineral mix	10	10	10	10	10
Vitamin mix	10	10	10	10	10
Choline bitartrate	2	2	2	2	2
Perilla oil	-	-	63	-	-
Olive oil	-	-	-	63	-
Safflower oil	-	-	-	-	63
Energy source	-	-	-	-	-
From fat (%)	10	60	60	60	60
From carbohydrate (%)	70	20	20	20	20
From protein (%)	20	20	20	20	20
Energy (kcal/g)	3.8	5.2	5.2	5.2	5.2

Mice were divided into 5 groups (n = 8); LFD, low-fat diet; HFD, high-fat diet; HPO, high-diet supplemented with perilla oil; HOO, high-fat diet supplemented with olive oil; HSO, high-fat diet supplemented with safflower oil.

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abdominal fat region from lumbar vertebrae 1 to 5 was analyzed, micro-CT images were captured, and total fat volume was analyzed by CT analyzer, Skyscan software (Konitch, Belgium).

Biochemical parameters

The mice were sacrificed after 12 h of fasting at the end of the experimental period. The length of the colon was measured (from cecum to rectum) using a fixed ruler. The macroscopic score was assessed according to the scale; 0 - no ulcer or inflammation, 1 - ulceration with local hyperemia, 2 - ulceration without hyperemia, 3 - ulceration and inflammation at one site, 4 - two or more sites with ulceration and inflammation, and 5 - ulceration extending more than 2 cm.

Serum was separated by centrifugation at 1,500 ×g for 15 min at 4°C and was stored at –72°C until analysis. Tissues were immediately frozen in liquid nitrogen and stored at –72°C. Commercially available kits were used to analyze the levels of serum triglyceride (TG), total cholesterol (TC), and high-density lipoprotein-cholesterol (HDL-C; Asan Pharmaceutical Co, Korea). Leptin and adiponectin levels in serum and proinflammatory cytokine such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, and IL-6 levels in the colon were measured using ELISA kits (R&D Systems, Cambridge, MA, USA).

Fecal microbial culture

Approximately 0.1 g of mice feces was collected in sterilized tubes and diluted 10-fold with autoclaved phosphate-buffered saline (PBS). The samples were further serially diluted 10-fold. For analyzing *Enterobacteriaceae*, the samples were inoculated in desoxycholate agar plates and cultured aerobically for 24 h at 37°C. For *Bifidobacteria*, the samples were plated onto BL agar plates and cultured anaerobically for 48 h at 37°C. The agar powders were purchased from MB cell, Seoul, Korea.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) and western blot

To check the expression of genes associated with obesity and colon inflammation, qRT-PCR was performed using liver, epididymal fat, and colon tissue. The mRNA was isolated from liver tissues using the TRI method and from colon using QIAGEN kit. After checking the purity, cDNA was synthesized using the PrimeScript RT master mix and qRT-PCR was performed. Sequences of the primers used in the study are given in **Table 2**. Inflammatory marker proteins, such as phospho-p65 (p-p65), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) in the colon, were measured using western blot. Tissues were lysed in Radioimmunoprecipitation assay buffer, centrifuged (4°C, 12,000×g, 15 min), and the supernatant was collected. After checking the protein concentrations, the samples were matched to the same concentration and heated at 95°C after mixing with 5X protein buffer. The proteins were electrophoresed on a 10–12% sodium dodecyl sulfate-polyacrylamide gel and then transferred onto a polyvinylidene difluoride membrane for blotting.

Statistical analysis

Statistical differences were analyzed using one-way Analysis of Variance in SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Significance was considered at $P < 0.05$, using Duncan's multiple range test. Values are expressed as mean ± standard deviation and values with different superscripts indicate significant difference between groups.

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Table 2. Primer sequence of the genes used in the present study

Gene name	Primers	Sequence (5′–3′)
PPARγ	Forward	GTG CCA GTT TCG ATC CGT AGA
	Reverse	GGC CAG CAT CGT GTA GAT GA
FAS	Forward	AGGGGTCGACCTGGTCCTCA
	Reverse	GCCATGCCCAGAGGGTGGTT
ACC	Forward	CCA ACA TGA GGA CTA TAA CTT CCT
	Reverse	TAC ATA CGT GCC GTC AGG CTT CAC
SREBP-1c	Forward	GAT CAA AGA GGA GCC AGT GC
	Reverse	TAG ATG GTG GCT GCT GAG TG
HMG-CoA reductase	Forward	CCG GCA ACA ACA AGA TCT GTG
	Reverse	ATG TAC AGG ATG GCG ATG CA
PPARα	Forward	GGA TGT CAC ACA ATG CAA TTC GCT
	Reverse	TCA CAG AAC GGC TTC CTC AGG TT
CPT-1α	Forward	AAA GAT CAA TCG GAC CCT AGA CA
	Reverse	CAG CGA GTA GCG CAT AGT CA
ACOX	Forward	CCC AAC TGT GAC TTC CAT T
	Reverse	GGC ATG TAA CCC GTA GCA CT
TNF-α	Forward	ACG GCA TGG ATC TCA AAG AC
	Reverse	GTG GGT GAG GAG CAC GTA GT
IL-6	Forward	AAC GAT GAT GCA CTT GCA GA
	Reverse	GAG CAT TGG AAA TTG GGG TA
IL-1β	Forward	GAC CTT CCA GGA TGA GGA CA
	Reverse	AGC TCA TAT GGG TCC GAC AG
IL-10	Forward	TAC CTG GTA GAA GTG ATG CC
	Reverse	CAT CAT GTA TGC TTC TAT GC
Claudin-1	Forward	TCT ACG AGG GAC TGT GGA TG
	Reverse	TCA GAT TCA GCA AGG AGT CG
ZO-1	Forward	ACC CGA AAC TGA TGC TGT GGA TAG
	Reverse	AAA TGG CCG GGC AGA ACT TGT GTA
MUC-1	Forward	CTG TTC ACC ACC ACC ATG AC
	Reverse	CTT GGA AGG GCA AGA AAA CC
β-actin	Forward	AGC CTT CCT TCT TGG GTA TGG
	Reverse	CAC TTG CGG TGC ACG ATG GAG

PPAR, peroxisome proliferator-activated receptor; *FAS*, fatty acid synthase; *ACC*, acetyl CoA carboxylase; *SREBP*, sterol regulatory element-binding protein; *CPT*, carnitine palmytoyl transferase; *ACOX*, acyl CoA oxidase; *TNF*, tumor necrosis factor; *IL*, interleukin; *ZO*, zonula occludens; *MUC*, mucin.

RESULTS

PO, OO, and SO significantly lowered body weight and organ weight

As shown in **Fig. 1**, all the HFD-fed groups showed significantly increased body weight compared to the LFD group. However, the three oil-supplemented groups showed significantly lower body weight than the HFD group from the seventh week of study (**Fig. 1A**) and a similar pattern was seen in the final body weight (**Fig. 1B**). The liver and epididymal fat weights were also significantly lowered in the oil-supplemented groups (**Fig. 1C** &and D). Micro-CT results (**Fig. 1E**) revealed that PO and SO significantly reduced the levels of fat, whereas OO showed a slightly lower abdominal fat mass compared to HFD (**Fig. 1F**).

PO, OO, and SO improved obesity-related serum biochemical parameters

As shown in **Fig. 2A**, serum TG level was significantly reduced in HPO and HSO, whereas the HOO showed a reducing tendency compared to the HFD group. There were no significant differences in the TC and HDL-C levels between the HFD and treatment groups (**Fig. 2B and C**). As shown in **Fig. 2D and E**, leptin and adiponectin levels were increased and decreased, respectively, in the HFD group. Interestingly, all the three oil-supplemented groups

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showed significantly reduced leptin levels. Furthermore, PO and SO significantly increased adiponectin levels whereas OO showed an increasing tendency compared with HFD.

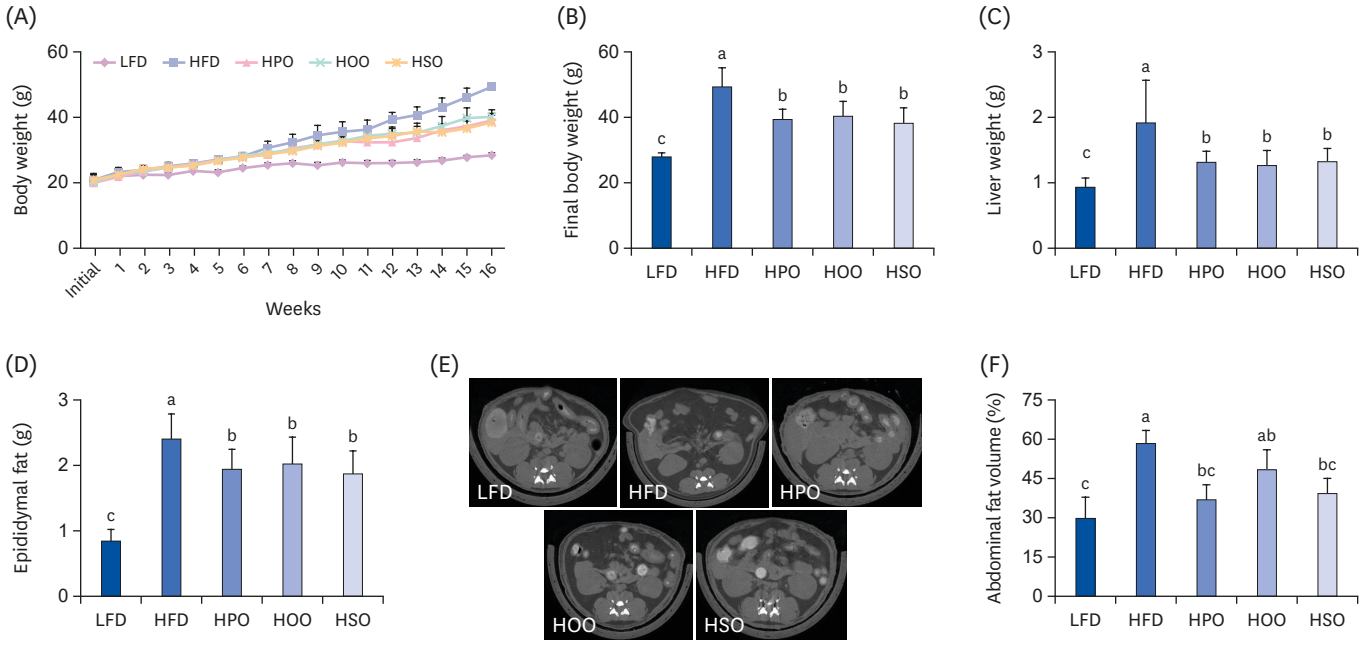

Fig. 1. Anthropometric parameters and micro-CT analysis. Body weight for the whole experimental period (A), final body weight (B), liver weight (C), epididymal fat (D), and (F) percentage of abdominal fat volume. Values are shown as mean ± SD. Values with different superscripts (a, b, c) letters are significantly different among groups. The micro-CT image (E) of abdominal region (dark grey portions indicate fat tissue). Mice were divided into 5 groups (n = 8); low-fat diet (LFD); high-fat diet (HFD); high-diet supplemented with perilla oil (HPO); high-fat diet supplemented with olive oil (HOO); high-fat diet supplemented with safflower oil (HSO).
CT, computed tomography.

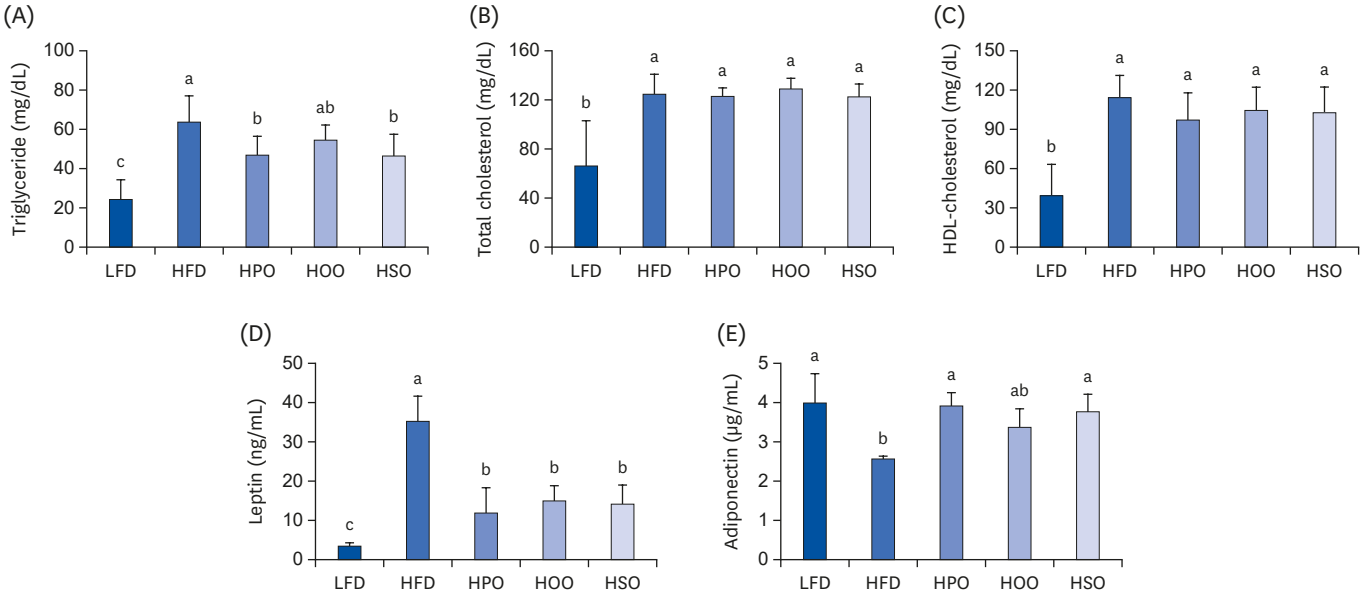

Fig. 2. Serum lipids and adipokine levels. (A)Triglyceride, (B) total cholesterol, (C) high-density lipoprotein-cholesterol, (D) leptin, and (E) adiponectin. Values are shown as mean ± SD. Values with different superscripts (a, b, c) letters are significantly different among groups. Mice were divided into 5 groups (n = 8); low-fat diet (LFD); high-fat diet (HFD); high-diet supplemented with perilla oil (HPO); high-fat diet supplemented with olive oil (HOO); high-fat diet supplemented with safflower oil (HSO).

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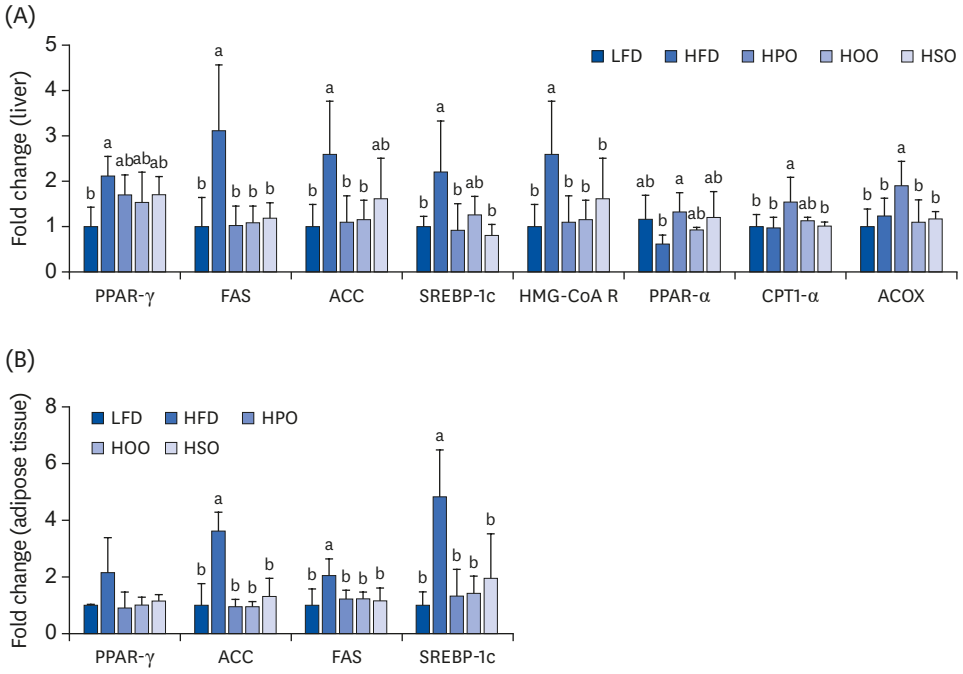

Fig. 3. The mRNA expression levels in (A) liver and (B) adipose tissue. Values are shown as mean ± SD. Values with different superscripts (a, b) letters are significantly different among groups. Mice were divided into 5 groups (n = 8); low-fat diet (LFD); high-fat diet (HFD); high-diet supplemented with perilla oil (HPO); high-fat diet supplemented with olive oil (HOO); high-fat diet supplemented with safflower oil (HSO).
PPAR, peroxisome proliferator-activated receptor; FAS, fatty acid synthase; ACC, acetyl CoA carboxylase; SREBP, sterol regulatory binding protein; HMG-CoA R, 3-hydroxy-3-methyl-glutaryl-CoA reductase; CPT, carnitine palmitoyl transferase; ACOX, acyl CoA oxidase.

PO, OO, and SO improved the gene expression of obesity-related markers in liver and adipose tissue

As shown in **Fig. 3**, the expression levels of genes associated with lipogenesis such as peroxisome proliferator-activated receptor gamma (*PPARγ*), fatty acid synthase (*FAS*), acetyl CoA carboxylase (*ACC*), sterol regulatory binding protein 1c (*SREBP1c*), and 3-hydroxy-3-methyl-glutaryl-CoA reductase (*HMG-CoA reductase*) were significantly increased in the liver and adipose tissue of HFD compared to LFD. Treatment with all three oils reduced the expression levels of these genes. On the other hand, the expression levels of hepatic lipid catabolic genes such as *PPARα*, carnitine palmitoyl transferase 1 alpha (*CPT-1α*), and acyl CoA oxidase (*ACOX*) were significantly upregulated in HPO compared to HFD, HOO, and HSO. Altogether, these results suggest that PO, OO, and SO exert anti-obesity effects by downregulating lipid synthesis and upregulating β-oxidation.

PO, OO, and SO improved colon inflammation

The colon length of the HFD group was significantly reduced compared to that of LFD. However, the colon length of PO, OO, and SO-supplemented groups was higher than that of the HFD (**Fig. 4A**). Similarly, the macroscopic score was also lower in the treatment groups than in the HFD group (**Fig. 4B**). Treatment with PO significantly reduced the levels of TNF-α (**Fig. 4C**), IL-6 (**Fig. 4D**), and IL-1β in the colon (**Fig. 4E**). However, OO and SO significantly reduced IL-6 while showing a reducing tendency in the TNF-α and IL-1β levels.

As shown in **Fig. 5A and B**, PO significantly increased the number of *Bifidobacteria* and reduced the number of *Enterobacteriaceae*. Treatment with OO and SO did not affect the number of

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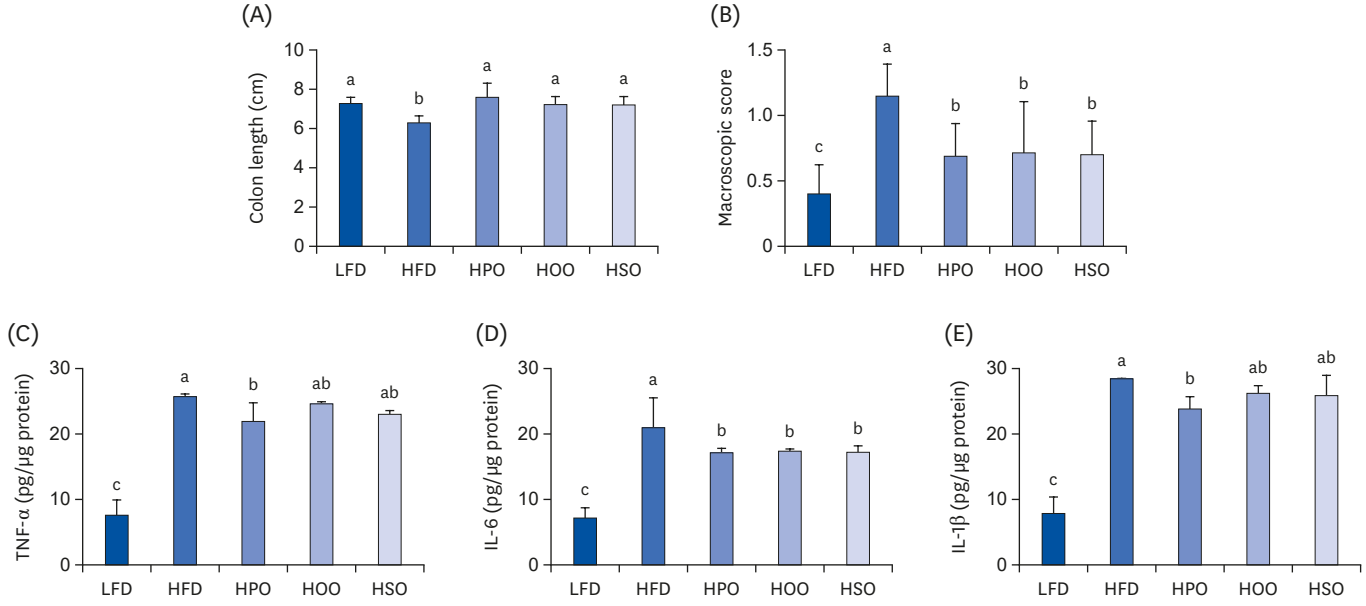

Fig. 4. Inflammation markers in the colon. (A) colon length, (B) macroscopic score, (C) TNF-α, (D) IL-6, and (E) IL-1β. Values are shown as mean ± SD. Values with different superscripts (a, b, c) letters are significantly different among groups. Mice were divided into 5 groups (n = 8); low-fat diet (LFD); high-fat diet (HFD); high-diet supplemented with perilla oil (HPO); high-fat diet supplemented with olive oil (HOO); high-fat diet supplemented with safflower oil (HSO).
TNF-α, tumor necrosis factor alpha; IL, interleukin.

Bifidobacteria; instead, the number of *Enterobacteriaceae* was reduced compared to the HFD group. Endotoxin levels were significantly reduced in all the three oil- supplemented groups, as shown in **Fig. 5C**.

As shown in **Fig. 6A**, the expression levels of proinflammatory markers such as *TNF-α*, *IL-6*, and *IL-1β* in the colon tissue were significantly increased in the HFD compared to that of LFD. Treatment with PO significantly attenuated these increases in proinflammatory marker levels while increasing the expression of the anti-inflammatory marker *IL-10*. Furthermore, the mRNA expression levels of the tight junction proteins *claudin-1* and zonula occludens-1 (*ZO-1*) were significantly upregulated in the PO-supplemented group. Although not significant, the gene expression of mucin-1 (*MUC-1*) was higher than the HFD group. The gene expression levels of *TNF-α*, *IL-6*, and *IL-1β* were significantly reduced in the OO and SO-supplemented groups, while the levels of *IL-10* were increased. Additionally, the levels of tight junction proteins and *MUC-1* showed an increasing tendency in the SO-supplemented group whereas OO did not show any effect on these markers. The protein expressions

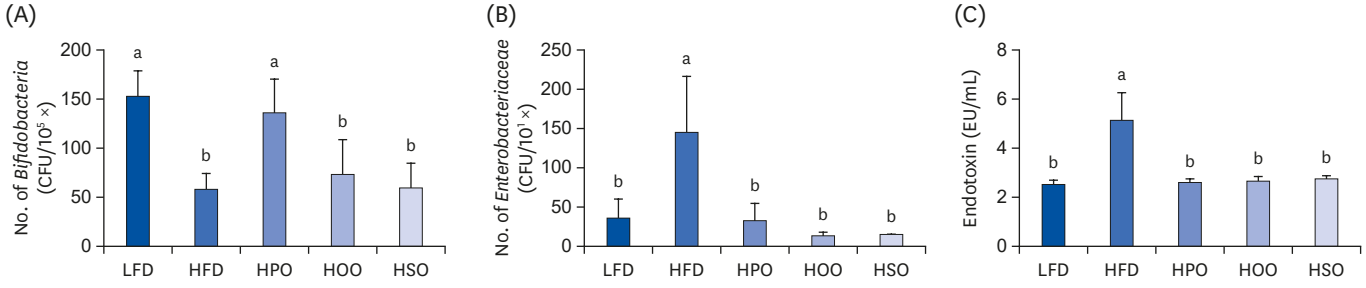

Fig. 5. Number of *Bifidobacteria* (A) and *Enterobacteriaceae* (B) in the feces and serum endotoxin levels (C). Values are shown as mean ± SD. Values with different superscripts (a, b) letters are significantly different among groups. Mice were divided into 5 groups (n = 8); low-fat diet (LFD); high-fat diet (HFD); high-diet supplemented with perilla oil (HPO); high-fat diet supplemented with olive oil (HOO); high-fat diet supplemented with safflower oil (HSO).

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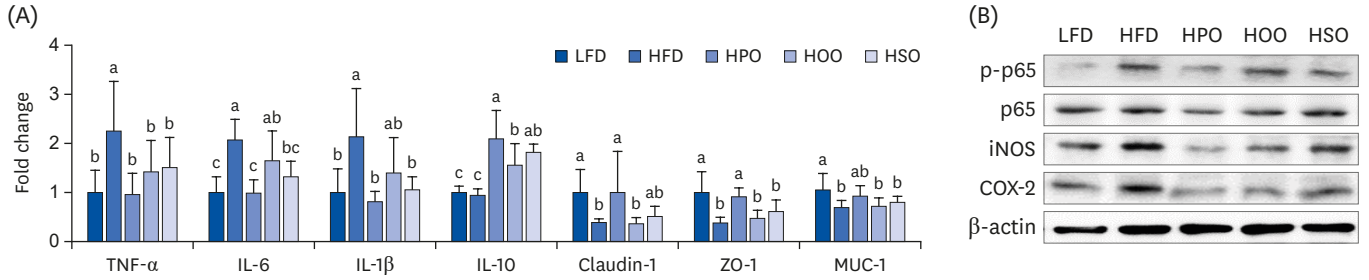

Fig. 6. The expression levels of inflammatory markers in colon. (A) mRNA expression using PCR, (B) protein expression using western blot. Values are shown as mean ± SD. Values with different superscripts (a, b, c) letters are significantly different among groups. Mice were divided into five groups (n=8); low-fat diet (LFD); high-fat diet (HFD); high-diet supplemented with perilla oil (HPO); high-fat diet supplemented with olive oil (HOO); high-fat diet supplemented with safflower oil (HSO).
p-p65, phospho-p65; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; TNF-α, tumor necrosis factor alpha; IL, interleukin; ZO, zonula occludens; MUC, mucin.

analyzed using western blot also showed that all the oil-treated groups significantly reduced the levels of p-p65, iNOS and COX-2. Compared to the HFD, HPO showed lower levels of proinflammatory markers, followed by HSO and HOO.

DISCUSSION

Obesity-associated complications contribute to increased economic burden in both developed and developing countries. Dietary and behavioral interventions are the preliminary steps toward managing obesity and related diseases [15]. Compared with unsaturated fat diet, saturated fat diet contributes to obesity and hepatic steatosis, and it can increase dietary fat flow to the intestine resulting in changes in the gut microbiota [16]. Reports have shown that replacing saturated fat with PUFA can reduce the risk of cardiovascular disease in humans [17]. Furthermore, long-term intake of omega-3 fatty acids reduces the risk of ulcerative colitis [18]. Oils are an unavoidable part of every cuisine, and each oil differs in its fatty acid composition. In this study, we aimed to investigate the effect of three mainly used dietary oils; PO, OO, and SO in HFD-induced obesity and colon inflammation.

In the present study, compared with HFD, supplementation with PO, OO, and SO improved body weight, organ weights, serum TG, leptin, and adiponectin levels. Interestingly, in the analysis of abdominal volume using micro-CT, HPO and HSO showed more pronounced effects than HOO. In some previous reports, PO has been shown to lower serum TG, TC, and low-density lipoprotein-cholesterol levels [19,20]. In the present study, PO lowered serum TG levels without affecting serum TC levels. According to a previous report, OO has hypolipidemic activity [21]. In the present study, compared with HFD, HOO group showed reduced TG levels, however TC level was not significantly different.

High leptin and low adiponectin levels are characteristic of obesity. Adiponectin contributes to β-oxidation by mediating the phosphorylation of AMP-activated protein kinase (AMPK) and activating *PPARα* [22]. PO suppressed leptin levels and increased adiponectin levels, which might have been reflected in the increased gene expression levels of β-oxidation in the HPO group. The results of our study showed that each of the oils exerts lipid-lowering effects and can improve obesity.

Supplementation with PO downregulated the expression of lipogenic genes, such as *PPARγ*, *FAS*, *ACC*, *SREBP1c*, and *HMG-CoA reductase* and upregulated the expression of *PPARα*, the

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transcription factor that increases the expression of genes related to β-oxidation. The observed pattern was similar to that of mRNA expression of lipogenic genes in adipose tissue. The results of a previous study also showed that PO improves lipid metabolism in the liver by phosphorylating ACC via activation of the AMPK pathway in HFD-fed mice [23]. Furthermore, Zhang *et al.* [24] reported that PO induces lipid oxidation in rats by increasing *PPARα* and *CPT-1α*. Treatment with OO and SO also showed downregulation of lipogenic genes and showed a tendency to increase β-oxidation-related genes. A previous study showed that high oleic acid SO increase the expression of hepatic *PPARα* target genes and decrease the expression *SREBP-1c*, *ACC* and *FAS* in rats [10]. Altogether, our results suggest that PO, SO, and OO ameliorate HFD-induced obesity possibly by reducing lipogenesis and increasing β-oxidation.

Many recent researches have shown the association between HFD and low-grade inflammation [25-27]. Chronic ingestion of HFD causes changes in the microbial population, which results in increased production of LPS. This in turn increases the production of proinflammatory cytokines such as TNF-α, IL-6, and IL-1β, and if this condition persists, it can lead to damage of the layers of intestinal barrier. Excess LPS is also known to affect the permeability of the intestinal barrier by disrupting tight junction proteins. Translocation of LPS into the systemic circulation can stimulate inflammatory responses in different tissues, and this is the starting point for several complications associated with obesity such as insulin resistance [6,28]. In the present study, PO, OO, and SO improved the inflammatory conditions by lowering proinflammatory cytokine levels and prevented colon shortening. Interestingly, PO exerted better improvement effects than OO and SO, as evident from the significantly reduced TNF-α and IL-1β levels. PO also increased *Bifidobacteria*, while OO and SO did not show any effect. However, all three oils lowered the numbered of *Enterobacteriaceae*, which is responsible for the reduced serum endotoxin level.

The expression levels of genes associated with colon inflammation and tight junction proteins showed that PO exerts a better protective effect than OO and SO. The mRNA expression levels of proinflammatory cytokines were downregulated by PO, OO, and SO supplementation. However, the expression of tight junction proteins, such as *claudin-1* and *ZO-1*, was increased only in HPO compared to HFD. Although HSO showed an increasing tendency in terms of *claudin-1* expression, HPO showed more pronounced expression levels. The expression levels of proteins such as p-p65, iNOS, and COX revealed that PO, OO and SO significantly reduced inflammatory markers in the colon compared to HFD. Interestingly, compared with OO and SO, PO showed a markedly higher inhibiting effect on NF-κB activation, as evident by the reduced p-p65 levels. A previous report on a TNBS-induced colitis model showed that ALA suppressed NF-κB activation as well as reduced proinflammatory cytokines and markers of oxidative stress in the colon [29]. ALA being the major compound in PO, although we used HFD-induced colon inflammation mice model, our study results were similar. A recent study on OO showed that it reduced the expression of *TNF-α* gene expression in the colon; however, although it showed a hypocholesterolemic effect, it did not show protective effect against colon inflammation in HLA-B27 transgenic rats, which spontaneously develop immune-mediated chronic intestinal inflammation [30]. The results of this study partially agree with those of the present study because OO reduced the expression of inflammatory markers in the colon and improved the colon length and macroscopic score. The difference in the mice models used might be a reason for variations in the results.

The fatty acid composition analysis of the three edible oils used in this study showed that PO contained a high amount of ALA (59.7 g/100 g), the precursor of EPA and DHA. Both OO and

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SO consisted of a high amount of oleic acid (71.2 g and 72.4 g per 100 g oil, respectively). The ratio of SFA: USFA was the same (1:10) in PO and SO. However, the ratio of MUFA: PUFA was higher in SO (5:1) compared to PO (1:5). Though OO and SO have a similar amount of MUFA, the ratio of SFA: USFA and MUFA: PUFA in OO were 1:5 and 10:1, respectively. There are two kinds of SO available in the market, one is high-oleic acid SO and the other is high-linoleic acid SO. Some previous researches have shown that high MUFA containing diets with a low SFA: USFA may ameliorate obesity [31,32]. In the present study, we also found that high-oleic acid SO that has a low SFA: USFA ratio showed less fat accumulation compared to OO which has a high SFA: USFA ratio.

To summarize, we investigated the effect of PO, OO and SO, oils with different fatty acid compositions, on HFD-induced obesity and colon inflammation. HFD contains high amounts of saturated fat whereas PO, SO, and OO contain higher amounts of unsaturated fats. Previous reports have shown that a low saturated fat: unsaturated fat ratio is beneficial for health [33]. In our study, we observed that HPO, HOO, and HSO showed lower levels of inflammatory markers, indicating that a diet containing a low ratio of saturated to unsaturated fatty acids is effective in reducing colon inflammation. Furthermore, PO showed a more pronounced effect on the improvement of colon inflammation when compared to OO and SO. These results emphasize the importance of replacing saturated fats with unsaturated fats, especially PUFA-rich perilla oil in consideration of a healthy dietary pattern.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1

Fatty acid composition of the oils used in the present study

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