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# Policosanol Reduces Blood Cholesterol Levels by Inhibiting Sterol Regulatory Element-binding Proteins-1c and Fatty Acid Synthase

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The underlying action of policosanol in lowering cholesterol level has not yet been clearly elucidated. Several recent studies have suggested that sterol regulatory element-binding proteins (SREBP)-1c play a role in the regulation of cholesterol synthesis via the fatty acid synthesis pathway. To date, no study has evaluated the effects of policosanol on SREBP-1c-mediated fatty acid synthesis. Therefore, this study aimed to investigate whether the SREBP-1c-mediated fatty acid biosynthetic pathway is associated with the cholesterol-lowering effects of policosanol. Seven-week-old C57BL/6 male mice were randomly divided into 7 groups (n=7 per group) and treated for 8 weeks as follows: 1) normal diet (normal control), 2) high-fat diet (HFD), 3) HFD+ethanol (Pol-0), 4) HFD+policosanol 1 mg/kg (Pol-1), 5) HFD+policosanol 2 mg/kg (Pol-2), 6) HFD+policosanol 4 mg/kg (Pol-4), and 7) HFD+ simvastatin 50 μg/kg (positive control). Policosanol and simvastatin were administered at the same time every day while maintaining the HFD. Body weight and food intake were measured weekly for 8 weeks. After 8 weeks, serum cholesterol levels were measured, histological analysis was carried out, and the expressions of SREBP-1c and fatty acid synthase (FAS) in the liver tissues were examined. Policosanol reduced body weight and the amount of food intake in a dose-dependent manner. Serum cholesterol levels were significantly lowered in the Pol-1 and Pol-4 groups. The expression of SREBP-1c and FAS was also significantly decreased in the Pol-4 group. These results suggest that the cholesterol-lowering effects of policosanol can occur due to the inhibition of the expression of SREBP-1c and FAS.

**Key words :** Blood cholesterol, fatty acid synthase, policosanol, SREBP-1c

## Introduction

Cholesterol is an essential component of cellular membranes and is the precursor for the production of steroid hormones, vitamin D, and bile acid. However, abnormally high levels of cholesterol in the blood are a significant risk factor for metabolic diseases. Among the metabolic diseases, cholesterol is a known risk factor for cardiovascular disease (CVD) leading cause of morbidity and mortality worldwide [11, 14]. For this reason, lowering blood cholesterol levels have been a key area of research in the management of CVD.

Cholesterol is biosynthesized by 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and HMG-CoA reductase inhibitors such as statins have been effectively used as lipid lowering agents. However, the long-term use of these drugs has been associated with various side effects, including liver damage, neurological problems and rhabdomyolysis [3, 34]. Therefore, research has focused on new and safer therapeutic strategies to reduce blood cholesterol levels using natural compounds or medicinal plants [1, 16, 17, 19].

Policosanol is a mixture of high molecular weight aliphatic alcohols with a long chain (22-34 carbons) that can be obtained from various natural foods such as sugarcane (*Saccharum officinarum* L.) wax [13]. The cholesterol-lowering ef-

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fects of policosanol in *in vitro* and *in vivo* studies using various animal models, as well as in human have been documented in literature [6, 10, 21]. As a result, policosanol is currently approved in more than 25 countries as a cholesterol-lowering supplement [35].

However, the underlying mechanism of action of policosanol has not yet been clearly elucidated. Cholesterol synthesis is regulated by sterol regulatory element-binding proteins (SREBPs), a family of membrane-bound transcription factors that regulate lipid homeostasis invertebrate cells [5]. SREBPs are present as three isoforms, namely SREBP-1a, SREBP-1c, and SREBP-2. Cholesterol biosynthesis is largely regulated by two pathways. One is the cholesterol synthesis pathway through SREBP-2 and its target gene, HMG-CoA reductase, which is the rate-limiting enzyme in cholesterol synthesis. The other pathway is regulated by SREBP-1c, which preferentially activates genes of fatty acid and triglyceride synthesis [12, 27, 30]. To date, the main mechanism of the cholesterol-lowering action of policosanol is believed to be the inhibition of HMG-CoA reductase activity by 5' adenosine monophosphate-activated protein kinase (AMPK) phosphorylation [4, 22, 23].

Liver X receptors (LXR)-mediated activation of SREBP-1ctranscription is involved in both the transport and storage of cholesterol via oleate, which is the fatty acid favored for cholesteryl ester synthesis [25]. Triacylglecerides (TG) and phospholipids produced by the SREBP-1c-derived pathway stimulate low-density lipoprotein (LDL) receptor expression [8]. These results suggest the role of SREBP-1c in the regulation of cholesterol synthesis. Nevertheless, there are no studies evaluating the effects of policosanol on SREBP-1c-mediated fatty acid biosynthetic pathway. Therefore, this study aimed to investigate whether SREBP-1c-mediated fatty acid biosynthetic pathway is associated with the cholesterol-lowering effects of policosanol.

## Materials and Methods

### Animals

Seven-week-old C57BL/6 male mice were purchased from Hanil Bio Inc. (Koatech Honam Distributor, Jeonbuk, Republic of Korea) and housed in colony cages under a 12 hr light/dark cycle with free access to water and food in a specific-pathogen-free (SPF) animal facility at a temperature of 21±2℃ and relative humidity of 55±10%. The animal experiments were approved by the Committee for the Care and Use of Laboratory Animals at the Pusan National University Hospital [PNUH-2022-196] and carried out according to the Guide for the Care and Use of Laboratory Animals, funded the National Institutes of Health, USA.

### Induction of hyperlipidemia in mice

After one week of adaptive feeding, the mice were randomly divided into two groups: normal control (normal diet, n=7) and high-fat diet (HFD, n=42). The normal control group was fed a normal diet, and the HFD group was fed a 60% high-fat diet (Samtako Bio Korea, Osan, Republic of Korea) containing 60% fat content, 20% protein and 20% carbohydrates for four weeks to induce obesity and hyperlipidemia. Mice were allowed to freely eat food and drink water. After four weeks, the body weight and total cholesterol levels were measured to confirm whether obese and hyperlipidemic mice models had been established.

### Preparation of policosanol sample and treatment

Policosanol from *Saccharum officinarum L* was obtained from the US Pharmatech Inc. (Torrance, CA, USA) and dissolved in 99% ethanol and used at concentrations of 1, 2, and 4 mg/kg. The same amount of ethanol was taken without policosanol for the 0 mg/kg policosanol treatment.

A total of 49 mice (7 normal controls and 42 HFDs) were equally randomized into 7 groups (n=7 per group); 1) normal control, 2) HFD, 3) HFD+ethanol (Pol-0), 4) HFD+policosanol 1 mg/kg b.w. (Pol-1), 5) HFD+policosanol 2 mg/kg b.w. (Pol-2), 6) HFD+policosanol 4 mg/kg body weight (b.w.) (Pol-4), 7) HFD+simvastatin 50 μg/kg b.w. (Sim, positive control). Policosanol and simvastatin were administered at the same time every day while maintaining the HFD for 8 weeks. The normal group was fed only a normal diet without HFD or policosanol for 8 weeks. After 8 weeks, all mice were prepared for further analysis.

### Measurement of body weight gain and food intake efficiency

During the 8-week experiment period, weight gain and food intake were measured at weekly intervals at the same time of the day using an electronic scale. Body weight gain was calculated using the following formula: eight gain [g] = final body weight (g) - initial body weight (g) (body weight before the start of the experiment). The food intake efficiency ratio was calculated by the total weight gain (g)/total food intake (g) ×100.

### Collection of blood and tissue samples

At the end of the treatment, the mice were sacrificed by inhalation euthanasia using carbon dioxide ($CO_2$). Immediately, blood was collected directly from the heart and placed into tubes without anticoagulant to separate the serum. The tubes were centrifuged at 3,000 rpm for 15 min at 4℃, and the serum were stored at -196℃ until further analysis. The livers were dissected and weighed, and then a part of the liver tissues was fixed with a 4% formaldehyde solution for histological analysis, and the rest of the tissues were quickly stored in liquid nitrogen for the gene expression study.

### Histological examination of the liver

For hematoxylin and eosin (H&E) staining, the liver sections were fixed with 10% buffered formalin, embedded in paraffin, cut at a thickness of 5 μm and then stained with H&E. Images were captured using the ImageScope software (Aperio Technologies, Vista, CA, USA).

### Measurement of total serum cholesterol levels

After the enzyme mix was added to the separated plasma, the total cholesterol levels in the blood were measured by quantifying the absorbance at $OD_{570nm}$ using a Cholesteryl ester Colorimetric/Fluorometric Assay Kit (K603-100, BioVision, Seoul, Republic of Korea).

### Total RNA extraction and reverse transcriptase-polymerase chain reaction (RT-PCR)

The livers were ground and homogenized in a mortar using liquid nitrogen, and the total RNA was extracted from the homogenized tissue using a TRIzol reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol. Complementary DNA (cDNA) was synthesized from 1μgof total RNA using Moloney Murine Leukemia Virus Reverse Transcriptase (Elpis Biotech, Daejeon, Korea) and an oligo-dT for 1 hour at 42°C, and enzymes were inactivated by heating for 5 min at 95℃. The template cDNA was subjected to PCR amplification using gene-specific primers (Table 1). After PCR amplification, the expression of the target gene was confirmed using an agarose gel, and the relative gene expression was normalized to the expression level of glyceraldehyde-3-phosphate dehydrogenase.

### Western blotting

The liver tissue samples were weighed, homogenized with radioimmunoprecipitation assay lysis buffer (ProEX™ CETi lysis buffer; TransLab, Seoul, South Korea), and centrifuged at 14,000 rpm at 4℃ for 20 min and the supernatant was then collected and preserved at -196°C. After lysis, the liver protein concentrations were quantified using a PierceTM bicinchoninic acid Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). An equal amount of protein (50 μg) was separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred to a polyvinylidene difluoride membrane (Millipore Corporation, Bedford, MA, USA). The membranes were blocked with the ProNA™ General-block solution (Translab, Seoul, South Korea) for 1 hr and incubated with diluted primary antibody solution overnight at 4℃. After washing, the membranes were incubated with horseradish peroxidase-conjugated monoclonal goat anti-rabbit IgG (TransGen Biotech, Beijing, China) for 1 hr at room temperature. Signals were detected using a ProNA™ ECLOttimo Western Blot Detection Kit (Translab, Seoul, Republic of Korea). The protein bands were visualized by the Western-Light Chemiluminescent Detection System (Image Station 4000 MM Pro, XLS180, Kodak, USA).

### Statistical analysis

Data were expressed as mean ± SD and were obtained from three independent experiments. The Student's t test was used to compare the body weights, biochemical parameters and total cholesterol levels between the groups with the sig-

Table 1. Primers sequences used for real time PCR amplification

| Gene | Sequence (5'→3') | |
|---|---|---|
| | Forward | Reverse |
| SREBP-1C | TTGTGGAGCTCAAAGACCTG | TGCAAGAAGCGGATGTAGTC |
| SREBP-2 | TGGGAGAGYYCCCTGATTTG | GATAATGGGACCTGGCTGAA |
| FAS | AGGGGTCGACCTGGTCCTCA | GCCATGCCCAGAGGGTGGTT |
| HMGCR | TCCAGCGACTATGAGCGTGAA | AGGGGTCGACCTGGTCCTCA |
| GAPDH | CCCATGTTTGTGATGGGTGT | GTGATGGCATGGACTGTGGT |

SREBP-1C: sterol regulatory element-binding protein-1c, SREBP-2: Sterol Response Element Binding Protein 2, FAS: fatty acid synthase, HMGCR: 3-hydroxy-3 methylglutaryl-coenzyme A reductase, GAPDH: glyceraldehyde 3-phosphate dehydrogenase

nificance level set at $p<0.05$.

## Results

### Establishment of the hyperlipidemia mouse model with a high-fat diet

HFD-induced hyperlipidemia was confirmed by weekly measurements of body weight and blood cholesterol levels. The mean body weight before the start of the experiment was 25.4±0.5 g and 24.9±0.7 g in the normal control group and the HFD group, respectively, and there was no significant difference between the two groups. However, the mean body weights in the normal control group after 1 week, 2 weeks, 3 weeks, and 4 weeks were 26.9±1.2 g, 27.7±0.8 g, 28.6±0.8 g, and 28.7±0.5 g, respectively, whereas those in the HFD group were 26.6±1.1 g, 28.4±1.0 g, 30.1±0.9 g, and 32.1±0.9 g, respectively. In 4 weeks, HFD significantly increased body weight (29.3%, average of 7.3 g) compared to the normal control group (12.9%, average of 3.3 g) (Fig. 1A). The blood cholesterol levels in the HFD group were 277.1±0.6 mg/dL after 4 weeks, which was about 2.7 times higher than that of the normal control group at 100.0±0.2 mg/dL (Fig. 1B). These results suggest that the HFD treatment used in this study effectively induces obesity and hyperlipidemia by increasing blood cholesterol levels in mice.

### The effects of policosanol on body weight and food intake efficiency

To determine the effects of policosanol on the ratio of weight gain and food intake efficiency in hyperlipidemic mice, the body weight and food intake were monitored weekly after treatment with HFD and policosanol for 8 weeks. Starting from the baseline, body weights steadily increased in all 7 groups regardless of the HFD and policosanol treatment for 8 weeks (Fig. 2A, Table 2). However, the body weight increased by 54.8% and 44.6% in the HFD and Pol-0 groups, respectively, whereas body weight increased by 34.6 %, 26.8%, and 10.2% in the Pol-1, Pol-2, and Pol-4 groups, respectively. This result showed that treatment with policosanol reduced body weight gain in a dose-dependent manner

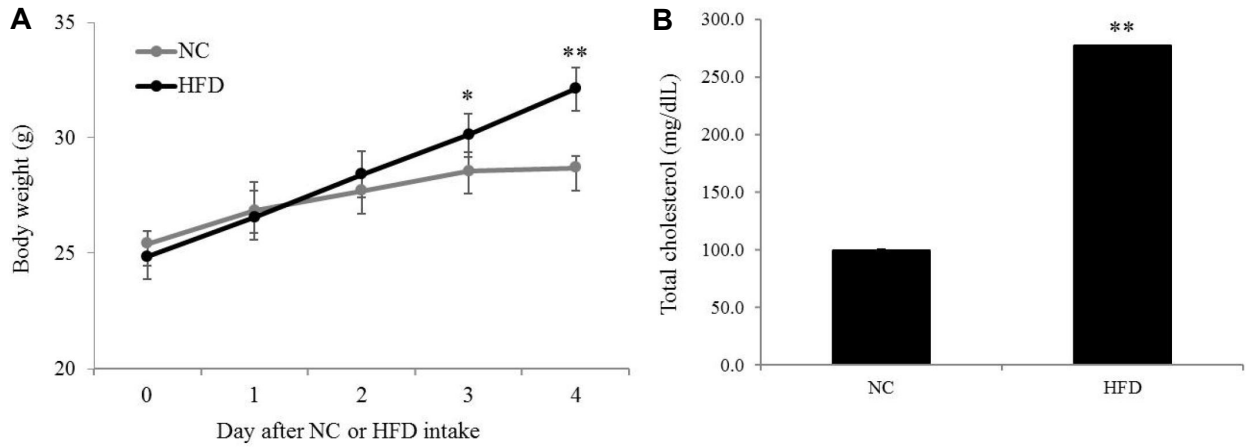


Fig. 1. Effects of high-fat diets on body weight gain and total serum cholesterol levels. Changes in body weight (A) and total serum cholesterol levels (B) in mice fed a normal diet (normal control, NC, n=7) and high-fat diet (HFD group, n=7) for 4 weeks. Data are presented as the mean ± SD. *$p<0.05$ and **$p<0.01$ (vs. NC group).

Table 2. Effect of policosanol on the body weight reduction

| Weeks | ND | HFD | HFD+Pol-0 | HFD+Pol-1 | HFD+Pol-2 | HFD+Pol-4 | HFD+Sim |
|---|---|---|---|---|---|---|---|
| 0 | 28.7 ± 0.5 | 32.1 ± 0.9 | 32.7 ± 2.5 | 32.1 ± 3.1 | 31.9 ± 2.4 | 31.9 ± 3.1 | 31.2 ± 2.4 |
| 1 | 29.0 ± 0.8 | 33.2 ± 3.8 | 33.0 ± 3.8 | 33.2 ± 3.0 | 32.9 ± 3.2 | 31.9 ± 2.6 | 31.7 ± 3.0 |
| 2 | 30.0 ± 1.0 | 36.4 ± 0.8 | 35.0 ± 3.8 | 34.4 ± 3.3 | 33.2 ± 4.0 | 31.0 ± 1.6 | 31.9 ± 3.0 |
| 3 | 30.4 ± 0.8 | 38.7 ± 1.4 | 37.3 ± 3.6 | 35.8 ± 3.5 | 34.6 ± 3.7 | 31.0 ± 1.7 | 32.5 ± 3.0 |
| 4 | 31.1 ± 1.3 | 42.3 ± 1.0 | 38.5 ± 3.1 | 36.3 ± 3.6 | 35.4 ± 3.8 | 32.0 ± 2.7 | 33.3 ± 2.9 |
| 5 | 31.4 ± 1.5 | 44.9 ± 1.5 | 40.3 ± 3.6 | 38.1 ± 4.0 | 36.8 ± 3.1 | 32.6 ± 2.1 | 35.0 ± 3.6 |
| 6 | 32.3 ± 1.6 | 46.0 ± 2.6 | 42.8 ± 3.9 | 40.1 ± 4.6 | 37.2 ± 3.2 | 33.9 ± 2.3 | 36.3 ± 3.7 |
| 7 | 32.3 ± 1.8 | 47.6 ± 2.1 | 45.0 ± 3.7 | 41.9 ± 5.3 | 39.2 ± 3.6 | 34.0 ± 5.0 | 38.3 ± 4.3 |
| 8 | 33.0 ± 2.6 | 49.7 ± 2.1 | 47.3 ± 3.8 | 43.2 ± 5.6 | 40.4 ± 3.4 | 35.1 ± 6.1 | 39.5 ± 4.7 |

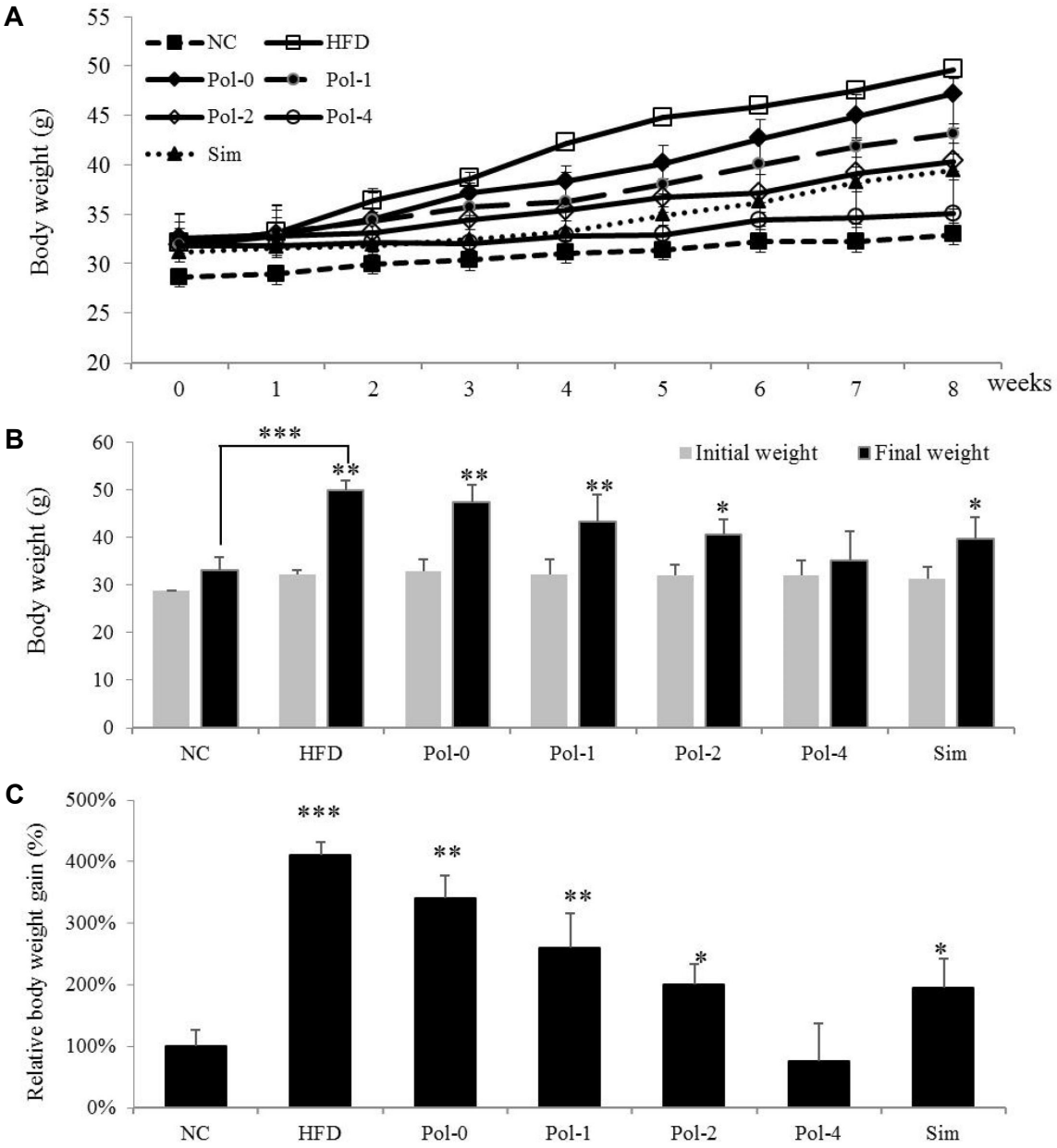


Fig. 2. Effects of policosanol on body weight reduction. (A) Comparison of the body weight of mice treated with a high-fat diet and policosanol for 8 weeks. Body weight was measured weekly. (B) Body weight prior to initiation of treatment and at the end of 8 weeks. (C) Relative weight gain (%) for 8 weeks. The present data were expressed as mean ± SD (n=7/group). Data represent the mean ± SD. *$p$<0.05 and **$p$<0.01(vs. the initial weight) and ***$p$<0.001 (vs. NC group).

(Fig. 2B). Specifically, the administration of 4 mg/kg of policosanol (Pol-4) significantly reduced the body weight gain to a level similar to that of the normal control group, and this reduction was more effective than that seen in the positive control group (Sim) ($p$<0.05) (Fig. 2C).

There was no significant difference in the amount of food intake between the normal control group and the HFD group during the experiment period. However, the food intake efficiency was significantly higher in the HFD group (6.03± 0.36%) compared to the normal control group (1.81±0.28%). Policosanol treatment reduced food intake efficiency ratio in a dose-dependent manner. Specifically, the administration of 4 mg/kg of policosanol (Pol-4 group) significantly lowered the food intake efficiency ratio compared to the positive control group as well as other groups (Table 3). These results indicate that policosanol treatment efficiently reduces the body weight gain caused by HFD ($p$<0.05).

Table 3. Effects of policosanol on food intake and efficiency

| | Body weight gain (g) | Food intake amount (g/day) | Food intake efficiency ratio (%) |
|---|---|---|---|
| ND | 7.6 ± 0.26 | 4.2 ± 0.3 | 0.95 ± 0.28 |
| HFD | 24.9 ± 0.21 | 4.1 ± 0.4 | 3.78 ± 0.36* |
| HFD + pol-0 | 22.1 ± 0.38 | 3.6 ± 0.3 | 3.26 ± 0.26* |
| HFD + pol-1 | 18.3 ± 0.56 | 3.2 ± 0.2 | 3.17 ± 0.22* |
| HFD + pol-2 | 16.1 ± 0.34 | 3.3 ± 0.2 | 2.62 ± 0.25*,# |
| HFD + pol-4 | 10.8 ± 0.61 | 3.0 ± 0.6 | 1.44 ± 0.58*,# |
| HFD + Sim | 15.4 ± 0.47 | 3.0 ± 0.1 | 2.96 ± 0.14*,# |

All values are mean±SD of 7 mice for each group. The food intake efficiency ratio was calculated by the total weight gain (g)/total food intake (g) × 100.* $p<0.001$ (vs. ND group), $^{\#}p<0.001$ (vs. HDF group).

### The effects of policosanol on liver weight and pathological changes in the liver

HFD significantly increased the weight of the liver by an average of 31.8% (2.9±0.3 g) compared to 2.2±0.1 g in the normal control group. However, policosanol treatment significantly reduced the liver weight that was increased by HFD. These weights were 1.8±0.3 g, 1.8±0.4 g, and 1.4±0.1 g in the Pol-1, Pol-2, and Pol-4 groups, respectively. Specifically, the reduction in liver weight seen with 4 mg/kg policosanol treatment in the Pol-4 group was as much as that seen in the positive control group (1.5±0.2 g) (Fig. 3A).

To determine whether the reduction in liver weight caused

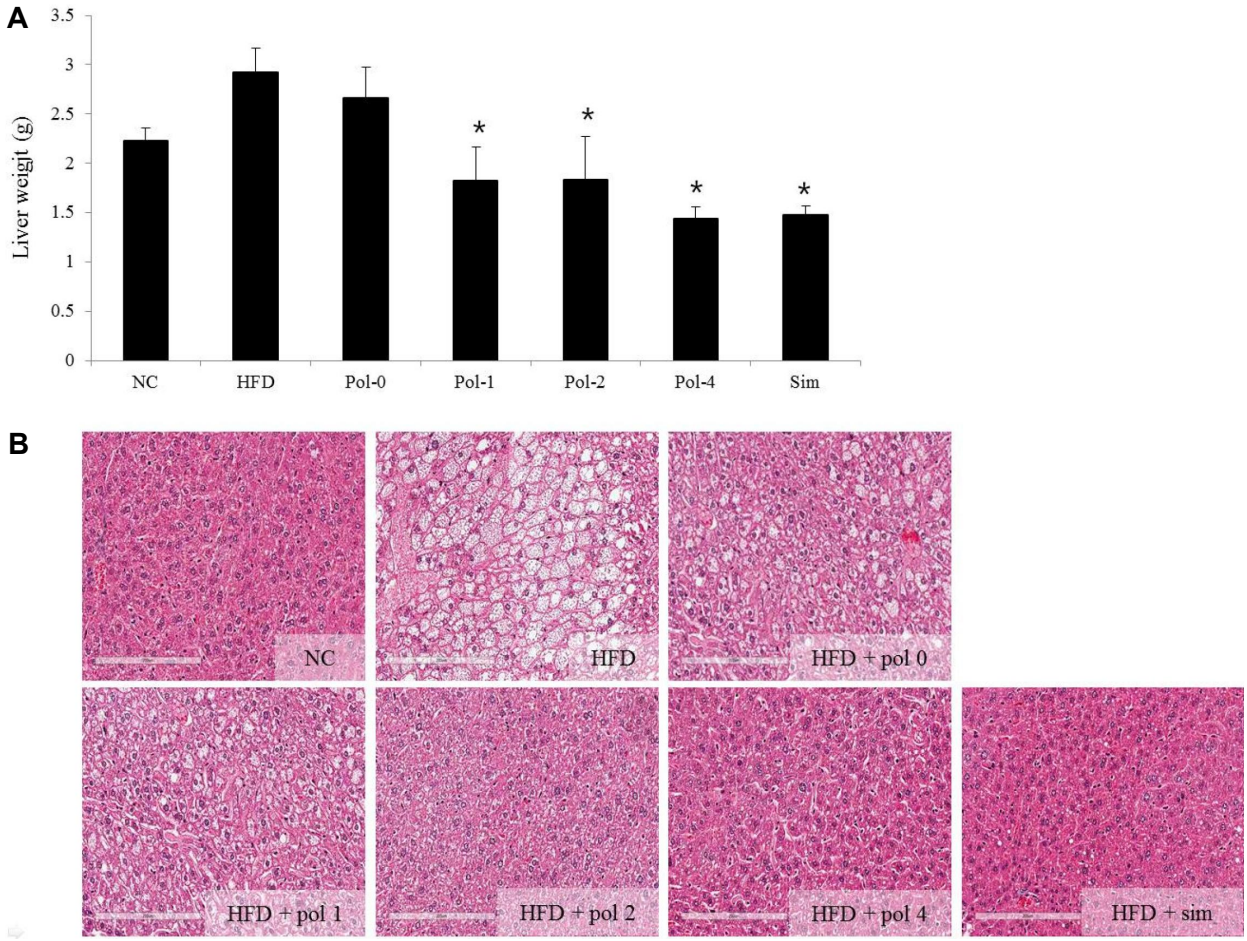


Fig. 3. Effects of policosanol on weight and pathological changes in the liver tissue. (A) The change in liver weight. (B) Histological changes in the liver tissue. The liver section was stained using H&E. The present data were expressed as mean ± SD (n=7/group). *$p<0.001$ (vs. HFD group).

by policosanol was related to fat accumulation in the liver tissue, a histological examination of the liver was performed by H&E staining. In the normal control group, the liver sections showed normal distinct hepatic cells, sinusoidal spaces, and a central vein. No lipid droplets were seen (Fig. 3B-NC). However, in the HFD group, the liver sections showed extensive lipid-droplet vacuoles throughout and hepatocyte morphology (Fig. 3B-HFD). On the other hand, policosanol treatment improved the morphology of the fatty liver to a normal appearance with a dose-dependent reduction in the size and number of lipid droplets (Fig. 3B-Pol-1, -2, -4). Specifically, in the 4 mg/kg policosanol (Fig. 3B-Pol-4) and simvastatin (Fig. 3B-Sim) treatments, there were no fat related changes and no loss of hepatocyte integrity, and liver tissues were restored to their original arrangement comparable to that of the normal control group.

### The effects of policosanol on total serum cholesterol levels

The study investigated whether the total serum cholesterol levels increased by HFD were decreased by policosanol administration. HFD significantly increased the total serum cholesterol levels to 566.9±33 mg/dL compared to 223.4±82 mg/dL in the normal control group ($p$<0.05). However, treatment with 2 mg/kg and 4mg/kg policosanol significantly reduced serum cholesterol levels to 472.5±21.1 mg/dL and 350.7±26 mg/dL, respectively (Fig. 4).

### The effects of policosanol on the expression of cholesterol synthesis-related genes

To understand the mechanism of action of policosanol in lowering blood cholesterol levels, the expressions of SREBP-1c, FAS, SREBP-2, and HMG-CoA reductase were investigated in the mRNA and protein levels by RT-PCR and the Western blot. The mRNA and protein expressions of all these genes were significantly increased in the HFD group compared to the normal control group ($p$<0.05). However, policosanol treatment decreased the expressions of these genes in a dose-dependent manner. Specifically, treatment with policosanol 4 mg/kg significantly reduced the expressions of these genes that had been increased by the HFD ($p$<0.01) (Fig. 5).

## Discussion

The cholesterol-lowering efficacy of policosanol in animals and humans has been extensively reported [2, 6, 20, 26, 35]. So far, most studies suggest that policosanol inhibits cholesterol synthesis by down-regulating the SREBP-2 and HMG-CoA reductase pathway [4, 21, 22, 32]. In the present study, s significant reduction in blood cholesterol levels was seen with policosanol. The cholesterol-lowering effects of policosanol in this study have been attributed to the down-regulation of SREBP-1c and FAS as well as SREBP-2 and HMG-CoA reductase gene expression. To our knowledge, this is the first report to suggest that policosanol can also modulate the SREBP-1c and FAS pathways in lowering cholesterol biosynthesis.

Numerous studies have shown that the continuous intake of HFD is effective in inducing hyperlipidemia with increase in blood cholesterol levels [24, 33, 37]. The present study also showed a significant increase in blood cholesterol levels in mice fed HFD, indicating that these mice are suitable ex-

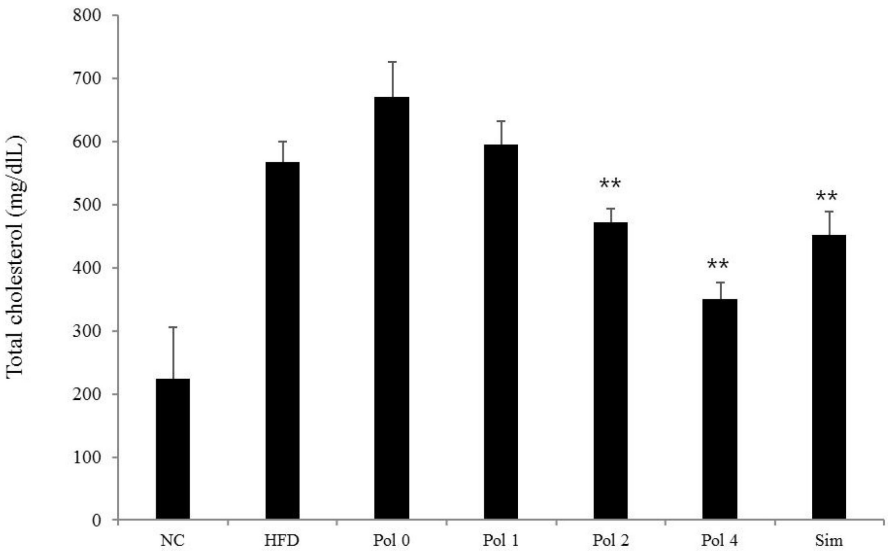


Fig. 4. Effects of policosanol on total serum cholesterol levels. The present data were presented as the mean ± SD. *$p$<0.05 (vs. NC group), **$p$<0.001 (vs. HFD group)

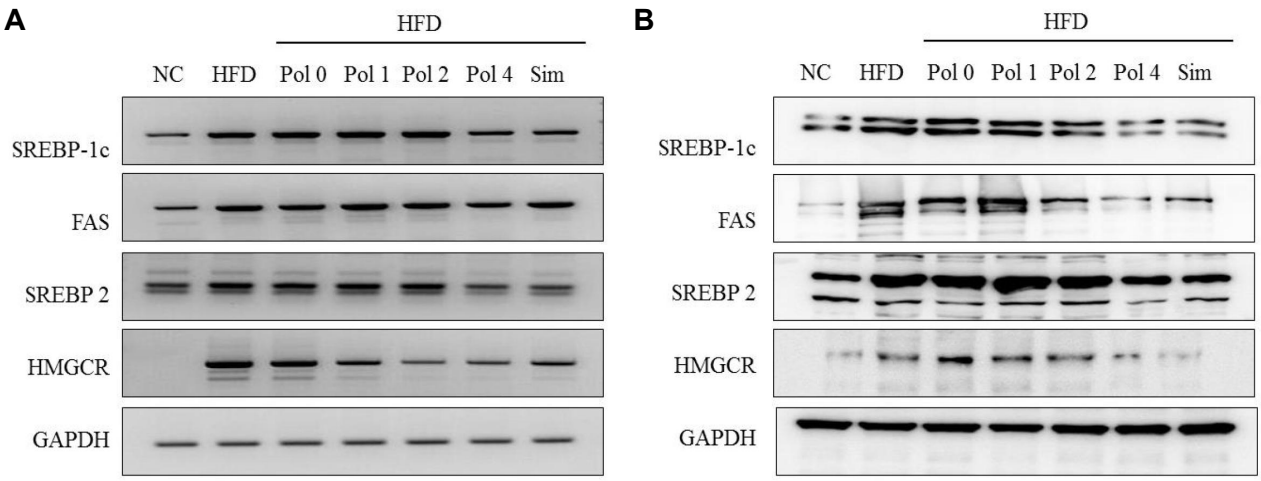


Fig. 5. Effects of policosanol on the expression of cholesterol synthesis-related genes in the liver. (A) mRNA expression levels for SREBP-1c, FAS, SREBP-2, HMGCR and GAPDH by RT-PCR. (B) Protein expression levels of SREBP-1c, FAS, SREBP 2, HMGCR and GAPDH by Western blot analysis. GAPDH was used as an internal control.

perimental model to study the effects of HFD and cholesterol-lowering medications on blood cholesterol. In this study, the administration of 4 mg/kg policosanol to HFD mice reduced blood cholesterol levels more effectively than simvastatin used as a positive control.

Obesity is a major risk factor for metabolic diseases, including cardiovascular diseases (CVD) [31]. Policosanol, along with octacosanol, has been reported to prevent obesity and metabolic disorders caused by HFD [29]. In the present study, a significant increase in body and liver weights as well as in blood cholesterol was seen in HFD-fed mice, and policosanol treatment effectively suppressed the HFD-induced weight gain by reducing the food intake and food intake efficiency ratio. These results suggest that policosanol can be very effective in the prevention of CVD and metabolic diseases through weight control and the reduction of blood cholesterol levels.

Excessive weight gain not only leads to the accumulation of adipose tissue, which further increases blood cholesterol levels, but also increases the weight of the liver along with an increase in the levels of TGs in the liver tissue [28]. A diet high-in fat not only increases the weight of the liver but can also cause fatty liver [36]. The present study also confirmed that HFD increased liver weight and caused abnormal hepatic lipid accumulation, and policosanol treatment decreased liver weight, lipid accumulation, and cholesterol levels in a dose-dependent manner.

Many studies have investigated the effects of policosanol on the serum levels of HDL, LDL, and TG in addition to total serum cholesterol levels, wherein policosanol treatment has been shown to increase HDL levels and decrease levels of LDL, TC, and total cholesterol through its action of HMG-CoA reductase and FAS which regulates their biosynthesis [6, 10, 15, 22]. One of the limitations of this study is that our study did not investigate the effects of policosanol on serum levels of LDL, HDL, and TG. Therefore, in this study, only the reduction in total cholesterol levels by policosanol was confirmed.

In conclusions, the present study revealed that policosanol can reduce blood cholesterol levels by down-regulating the expressions of FAS and SREBP-1c genes in addition to HMG-CoA reductase and SREBP-2 genes. Further, the study also showed that policosanol effectively reduces body weight, food intake, and fatty liver. Previous clinical and post-marketing surveillance studies have reported that policosanol is safe, well tolerated, and free from drug-related adverse events [7, 9, 18]. These results can help researchers understand the mechanism of action of policosanol in lowering blood cholesterol levels. The results of this study can find practical application in the development of policosanol supplements for improving blood circulation and reducing body fat.

## The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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## 초록 : Sterol regulatory element‐binding proteins-1c와 지방산 합성효소의 억제를 통한 폴리코사놀의 혈중 콜레스테롤 감소

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폴리코사놀의 콜레스테롤 감소 효과에 대한 기전은 아직 명확하게 규명되지 못하고 있다. 최근 몇몇 연구들은 지방산 합성 경로를 통한 콜레스테롤 합성의 조절에 sterol regulatory element-binding proteins (SREBP-1c)의 역할을 제시하였다. 그러나 현재까지 SREBP-1c에 의해 조절되는 지방산 합성에서 폴리코사놀의 효과에 대한 연구는 전무하다. 그러므로 본 연구의 목표는 SREBP-1c에 의해 매개되는 지방산 합성이 폴리코사놀의 콜레스테롤 감소 효과와 관계하는지를 조사하는 것이다. 7주령의 C57BL/6 수컷 생쥐를 7개 군(군당 7마리)으로 나누고 8주간 다음과 같이 처리하였다; 1) 정상식이군(정상 대조군), 2) 고지방식이군 (high-fat diet, HFD, 음성대조군), 3) 고지방식이+에탄올처리군(Pol-0), 4) 고지방식이+1 mg/kg 폴리코사놀처리군(Pol-1), 5) 고지방식이+2 mg/kg 폴리코사놀처리군(Pol-2), 6) 고지방식이+4 mg/kg 폴리코사놀처리군 (Pol-4),7) 고지방식이+simvastatin 50 μg/kg 처리군(양성 대조군). 폴리코사놀과 simvastatin은 고지방식이를 유지하는 동안 매일 동일 시간에 처리하였으며 체중과 음식섭취량은 8주 동안 매주 측정하였다. 8주 후, 혈중 콜레스테롤 수치를 측정하였으며, 간의 조직학적 분석과 SREBP-1c와 지방산의 발현을 조사하였다. 폴리코사놀은 농도-의존적으로 체중과 음식섭취량을 감소시켰다. 혈중 콜레스테롤 수치는 Pol-1과 Pol-4군에서 유의하게 감소하였으며, SREBP-1c와 FAS의 발현 역시 Pol-4 군에서 유의하게 감소하였다. 이러한 결과들은 폴리코사놀의 콜레스테롤 감소 효과가 SREBP-1c와 FAS의 발현 억제에 기인하여 일어날 수 있음을 시사한다.