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Effects of Long-Term Supplementation of Policosanol on Blood Cholesterol/Glucose Levels and 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Activity in a Rat Model Fed High Cholesterol Diets

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Abstract Policosanol is a well-defined nutraceutical for the management of blood cholesterol levels. The present study examined (i) the effect of policosanol supplementation on blood cholesterol and glucose levels and (ii) changes in hepatic cholesterol biosynthesis using 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) activity in Wistar rats fed high cholesterol diets. The Wistar rats were assigned randomly to high-cholesterol diets (1.25% cholesterol) with or without policosanol (8.0 mg/kg body weight) for 6 weeks. Compared with the control group, dietary treatment with policosanol resulted in a significant decrease of blood cholesterol (p<0.01), blood glucose (p<0.01), triglyceride (p<0.001), and low density lipoprotein-cholesterol levels (p<0.01) and HMG-CoA reductase activity (p<0.001) in the liver. These results indicate that policosanol decreases blood cholesterol levels by suppressing cholesterol biosynthesis via decrease of HMG-CoA activity. Policosanol has the potential to be developed into an effective dietary strategy for both postprandial hyperglycemia and hypercholesterolemia.

Keywords: policosanol, low density lipoprotein-cholesterol, blood glucose, HMG-CoA reductase, hypercholesterolemia

Introduction

Hypercholesterolemia is a disorder of the metabolism of dietary fats that affects approximately 31.7% of the total US population and results in great health care costs (1,2). Elevated cholesterol levels are a well-defined risk factor for cardiovascular diseases (CVD) and stroke.

Mevalonate, an important metabolite involved in cholesterol biosynthesis, is catalyzed by 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (EC 1.1.1.34). Because over 70% of the total body cholesterol is produced by *de novo* biosynthesis (3), inhibition of this enzyme can be a way to regulate fat metabolism because it will result in a significant reduction of blood cholesterol after consumption of a mixed fat diet. Therefore, HMG-CoA reductase inhibitors are widely prescribed to decrease blood cholesterol levels in humans and animals and are well documented (4).

Policosanol, a long-chain aliphatic alcohol (C26-C32) isolated from wax of sugar cane (*Saccharum officinarum* L.), was shown to decrease blood cholesterol levels in human and animal trials (5,6). Policosanol supplements have been approved as a cholesterol-lowering supplement in over 25 countries (7). Previous studies have demonstrated that policosanol could lower blood cholesterol via the promotion of AMP-kinase phosphorylation and HMG-CoA reductase in the liver of mice and hepatoma cells following intragastric administration (8,9). This phosphorylation decreases HMG-CoA reductase activity about 70-80% (9). Similar results indicating that policosanol treatments decrease hepatic cholesterol synthesis in rabbits and rats models have been reported (6,10,11). These results provide a solid biochemical mode of action to prove the ability of policosanol to lower cholesterol biosynthesis via the inhibition of HMG-CoA reductase and subsequent activation of AMP-kinase (8,9,12).

Activation of AMP-kinase is very important for the signaling of insulin, the body energy balance, and the metabolism of dietary glucose and fats (13,14). Metformin, one of the drugs for diabetes mellitus, is reported to decrease cholesterol/glucose levels via activation of AMP-activated protein kinase (AMPK) (15,16). Interestingly, policosanol has also been shown to increase the phosphorylation of

the principal AMPK, liver kinase B1 (LKB1), and this increase was slightly less than the increase caused by metformin (9). Additionally, the activation of AMPK through the use of long chain fatty acids has been demonstrated (17). Taken together, it is possible to manage both blood glucose and cholesterol levels using policosanol via AMP-kinase activation processes.

Therefore, in this study, the effect of supplementation of policosanol on the blood cholesterol and glucose management was evaluated using Wistar rats fed a high cholesterol diet. Policosanol was administrated for 42 days in Wistar rats. The effects of policosanol supplementation were compared with lovastatin, a HMG-CoA reductase inhibitor. Changes in blood glucose levels, total cholesterol, triglyceride contents, high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol, as well as HMG-CoA reductase activity were investigated.

Materials and Methods

Materials Lesstanol® Brand Policosanol (policosanol sugar cane wax alcohol) was donated by Garuda International, Inc. (Lemon Cove, CA, USA). The alcohol content was investigated by gas chromatography with flame ionization detector (62.3% of octacosanol, 13.3% of triacontanol, 9.2% of hexacosanol, 8.4% of dotriacontanol, 0.8% of tetracosanol, 1.5% of tetratriacontanol, 0.9% of heptacosanol, and 0.1% of nonacosanol). Total alcohol content was 96.5%. A high cholesterol diet (D12336; 75 g of casein, 130 g of soy protein, 2 g of DL-methionine, 275 g of corn starch, 150 g of maltodextrin 10, 30 g of sucrose, 90 g of cellulose, 50 g of soy bean oil, 75 g of cocoa butter, 35 g of coconut oil, 35 g of mineral mix (1), 5.5 g of calcium carbonate, 8 g of sodium chloride, 10 g of potassium citrate, 10 g of vitamin mix (2), 2 g of choline bitartrate, 12.5 g of cholesterol, 5 g of sodium cholic acid, and 0.1 g of FD&C red dye #40 in 1 kg of diet) was purchased from Raon Bio Inc. (Yongin, Korea). Two kits for measurement of total triglyceride and total cholesterol were obtained from Stanbio Laboratory (LiquiColor® Test series; Stanbio Laboratory, Boerne, TX, USA). Blood glucose measurement kits were obtained from Caresens (I-SENS, Anyang, Korea). HMG-CoA reductase assay kits (K588-100) were purchased from BioVison Inc. (Milpitas, CA, USA).

Animal and study design Wistar rats (5-week-old male) were obtained from Joongang Experimental Animal Co. (Seoul, Korea). Purina Pico #5053 diet (Oriental Bio. Co., Seongnam, Korea) was fed to all rats for one week. After 1 week, the diet was switched to the high-cholesterol diet D12336 (Raon Bio Inc.) for another 6 weeks. During the high-cholesterol diet D12336 administration for 6 weeks, policosanol and lovastatin were administered once a day (9-10 AM). Policosanol was suspended in a Tween 20-water vehicle (0.4% Tween 20 solution) and orally administered by gastric gavage (1 mL/kg). Three groups were used: a control group receiving the vehicle, and two treated groups receiving 0.008 and 0.006 g of policosanol and lovastatin/kg/day, respectively. Wistar male rats were single-housed in stainless cages with a 12 h light and dark cycle. The animal laboratory was maintained with 50±8% relative humidity. The number of Wistar rat in each group was 10. The experimental protocols were evaluated and approved by the Hannam University Institutional Animal Care and Use Committee (HNU-IACUC) (Approval number: HNU2015-0004). The rats were anesthetized with pentobarbital and killed, and then blood samples were obtained.

Blood analysis The glucose level in blood was measured with a glucose analyzer (CaresensII; I-SENS Inc.) by the glucose oxidase method. The assay protocols for total glyceride, total cholesterol, and HDL/LDL-cholesterol concentration in blood followed the method of Menedez *et al*. (6) using commercial kits (Liquicolor® test series; Stanbio Laboratory) and a semi-auto biochemistry analyzer (A6; Beijing Shinging Sun Technology Co., Ltd., Beijing, China).

HMG-CoA reductase activity The preparation of microsomes from rats' livers and the slow freeze-thaw procedure used to solubilize the enzyme have been previously described by Renu and Shrewsbury (18). Buffer A, which contained 0.2 M sucrose/0.05 M KCl/50 mM phosphate buffer (pH 7.0)/0.03 M EDTA, was used for the preparation of microsomes and Buffer B (Buffer A containing 10 rnM dithiothreitol) was used for the preparation of solubilized enzyme and in the subsequent purification. Briefly, the frozen microsomes were thawed at room temperature and gently homogenized with ground glass Potter-Elvehjem type glass homogenizers (Sigma-P7859; Sigma-Aldrich Co., St. Louis, MO, USA) in ice-cold Buffer B in a total volume of 240 mL. The homogenate was centrifuged at 105,000×*g* for 1 h and the supernatant referred to as Fraction I was collected. A repeat of the freezing-thawing homogenization procedure in a total volume of 120 mL and centrifugation gave the Fraction II supernatant. Both Fractions I and II were then combined and incubated at 37°C for 3 h. During this treatment, the solution became turbid and the insoluble components were removed by centrifugation at 34,000×*g* for 10 min. The clear supernatant that contained all the enzyme activity was harvested then used for further steps. The hepatic microsomal HMG-CoA reductase activity was measured using a kit (K588-100; BioVison Inc.).

Statistical analysis Results were presented as mean and standard deviation (mean±SD). Statistical analyses were performed by the statistical package "Statistical Package for Social Science (SPSS) ver. 10" (SPSS Inc., Chicago, IL, USA) program. Statistical significance of the data was tested by the Student's *t*-test and was defined as $p<0.05$.

Results and Discussion

Effect of policosanol on body weight Effects of policosanol

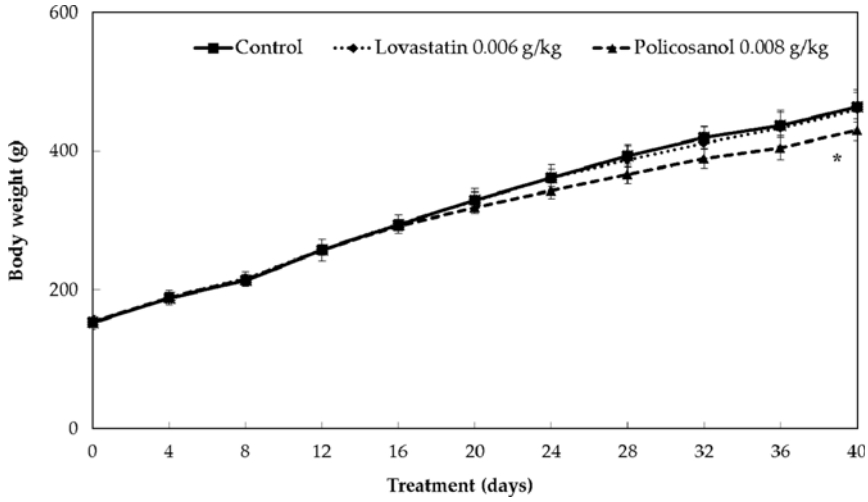

Fig. 1. Changes in body weight after administration of policosanol. Five-week-old male Wistar rats were fed a normal diet (Pico 5053) for 1 week. After 1 week, the diet was switched to a high-cholesterol diet (D12336) for another 6 weeks. During the high-cholesterol diet administration for 6 weeks, policosanol and lovastatin were administered once a day (9-10 AM). Policosanol was suspended in a Tween 20-water vehicle (0.4% Tween 20 solution) and was orally administered by gastric gavage (1 mL/kg). Three groups were used: a control group receiving the vehicle, and two treated groups receiving 0.008 and 0.006 g of policosanol and lovastatin/kg/day, respectively. Each point represents mean±standard deviation (SD) (n=10). Body weight levels were compared between control and treatment groups at each time point by unpaired Student's t-test (*p<0.05).

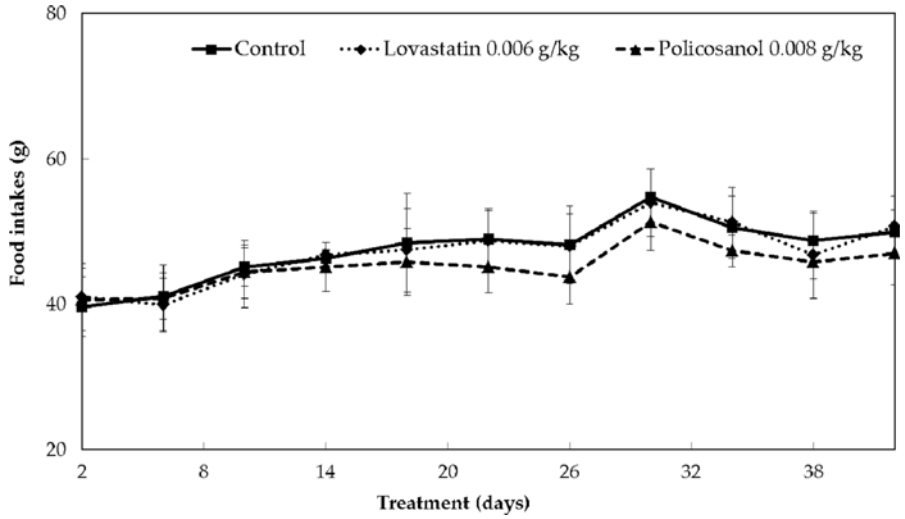

Fig. 2. Changes in food intake after the administration of policosanol. Five-week-old male Wistar rats were fed a normal diet (Pico 5053) for 1 week. After 1 week, the diet was switched to a high-cholesterol diet (D12336) for another 6 weeks. During the high-cholesterol diet administration for 6 weeks, policosanol and lovastatin were administered once a day (9-10 AM). Policosanol was suspended in a Tween 20-water vehicle (0.4% Tween 20 solution) and was orally administered by gastric gavage (1 mL/kg). Three groups were used: a control group receiving the vehicle, and two treated groups receiving 0.008 and 0.006 g of policosanol and lovastatin/kg/day, respectively. Each point represents mean±standard deviation (SD) (n=10). Body weight levels were compared between control and treatment groups at each time point by unpaired Student's t-test (*p<0.05).

supplementation were investigated in Wistar male rats for 42 days. The body weight in the policosanol-treated group was decreased significantly (p<0.05) compared with the lovastatin treatment and control groups after 42 days (Fig. 1). These significant changes in body weight between control and treatment groups (policosanol) were shown after 16 days of supplementation (Fig. 1). The positive control group treated by lovastatin and control group showed similar changes in food consumption while food intake was slightly decreased in the group treated by policosanol (Fig. 2).

Effects of policosanol supplementation on blood glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglyceride content The effects of treatment with policosanol for 42 days on blood glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride content, and liver/kidney weights were also investigated as shown in Table 1 and Fig. 3. Glucose levels in blood were

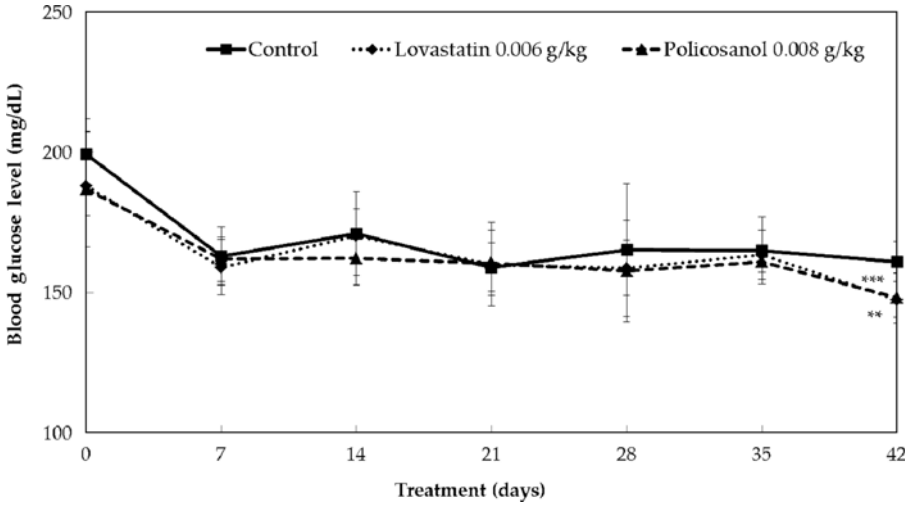

Fig. 3. Changes in blood glucose level after administration of policosanol. Five-week-old male Wistar rats were fed a normal diet (Pico 5053) for 1 week. After 1 week, the diet was switched to a high-cholesterol diet (D12336) for another 6 weeks. During the high-cholesterol diet (D12336) administration for 6 weeks, policosanol and lovastatin were administered once a day (9-10 AM). Policosanol was suspended in a Tween 20-water vehicle (0.4% Tween 20 solution) and was orally administered by gastric gavage (1 mL/kg). Three groups were used: a control group receiving the vehicle, and two treated groups receiving 0.008 and 0.006 g of policosanol and lovastatin/kg/day, respectively. Each point represents mean±standard deviation (SD) (n=10). Body weight levels were compared between control and treatment groups at each time point by unpaired Student's t-test (*p<0.05).

significantly decreased in the policosanol treatment group (p<0.01) similarly to the lovastatin treatment group (p<0.001) (Table 1 and Fig. 3). In the control group, the average glucose level in blood was 161.1 (mg/dL). Blood glucose levels in the policosanol and lovastatin groups were 148.2 (mg/dL) and 147.5 (mg/dL), respectively (Table 1). While total cholesterol level in the control group was 204.9 (mg/dL), the total cholesterol levels in the policosanol (p<0.01) and lovastatin treatment groups were 166.0 and 173.9 mg/dL, respectively (Table 1). Serum triglyceride levels of groups who were administered either policosanol or lovastatin were found to be not significantly different from each other (Table 1). HDL-cholesterol showed a tendency to increase with both policosanol and lovastatin supplementation; however, this finding was not statistically significant (Table 1). LDL-cholesterol, which is known to be associated with atherosclerosis and CVD, was significantly decreased in both policosanol (p<0.01) and lovastatin (p<0.01) treatment groups. Several *in vivo* studies showed that policosanol consumption has blood LDL-cholesterol lowering effects in *in vivo* studies (19) and in human clinical studies (20). Similar results have been reported suggesting that policosanol treatments decrease blood LDL-cholesterol levels in human clinical trials (21,22). Yanai *et al*. reported that policosanol had a LDL-cholesterol lowering effect (7).

Our findings indicate that policosanol could prevent body weight gain and cholesterol content induced by a cholesterol-enriched diet in a similar manner to the HMG-CoA reductase inhibitor, lovastatin (Fig. 1 and Table 1). However, recent studies have indicated that policosanol could not inhibit HMG-CoA reductase directly (9). Therefore, the HMG-CoA reductase-lowering effect seen in the

Table 1. Effects of policosanol and lovastatin treatment on various parameters in Wistar rats

Parameters	Wistar rats		
	Control	Lovastatin 0.006 g/kg	Policosanol 0.008 g/kg
Initial body weight (g)	153.50±9.58[1)]	154.50±8.01	154.50±7.78
Final body weight (g)	476.60±32.03	470.80±44.81	440.60±23.04*
Blood glucose (mg/dL)	161.07±7.10	147.50±6.36***	148.15±8.88**
Total cholesterol (mg/dL)	204.93±24.64	173.91±11.82**	166.04±22.32**
Triglyceride (mg/dL)	101.19±27.15	66.86±11.28**	35.53±8.41***
HDL-Cholesterol (mg/dL)	73.45±17.02	97.92±30.80	89.97±17.40
LDL-Cholesterol (mg/dL)	111.15±31.60	70.41±23.91**	70.43±23.13**
HMG-CoA reductase (Units/mg protein)	0.028±0.005	0.023±0.004*	0.017±0.003***
Liver (g)	31.75±5.78	32.04±4.68	28.66±3.02
Kidney (g)	3.37±0.34	3.23±0.51	3.09±0.34

[1)]Each point represents mean±SD (n=10). All parameters were compared between control and treatment groups at 42 day by unpaired Student's t-test (*p<0.05; **p<0.01; and ***p<0.001).

present study might be linked to HMG-CoA reductase activity, one of the key enzymes in cholesterol synthesis (9). Further specific enzyme activity assays and protein expression will be implemented to confirm the mechanism of action.

Changes in HMG-CoA reductase activity and liver/kidney weights HMG-CoA reductase is one of the rate-controlling enzymes in the mevalonate pathway that produces cholesterol from acetyl-CoA. In an NADPH-dependent reaction, HMG-CoA reductase reduces HMG-CoA to generate mevalonate and CoA. The enzyme is the target of a group of cholesterol-lowering drugs known as statins. Inhibition of HMG-CoA reductase induces expression of LDL receptors in the liver, which lowers plasma concentrations of cholesterol. Therefore, hepatic HMG-CoA reductase activity was evaluated after biopsy in all groups in the current study. The policosanol treatment group had the lowest activity (0.017 units/mg protein), followed by the lovastatin treatment group (0.023 units/mg protein), while the control group had the highest HMG-CoA reductase activity (0.028 units/mg protein) (Table 1). In order to evaluate the side effects of these 2 samples, liver and kidney weights were also measured after biopsy and control, policosanol, and lovastatin treatment groups had no significant differences in liver and kidney weights (Table 1). Our findings suggest that, in an animal model, policosanol has an suppressive effect against cholesterol production due to inhibition of HMG-CoA reductase.

Although several *in vivo* studies (19) as well as human clinical studies (8,21,22) have shown that policosanol consumption has a blood LDL-cholesterol lowering effect, we must not overlook other clinical reports in patients with hypercholesterolemia or combined hyperlipidemia that have shown that policosanol had no effect (23,24). Chen *et al*. (25) performed a systematic review and reported that policosanol induced a clinically significant decrease in the LDL/HDL ratio (4,596 patients from 52 clinical studies). This could be due to the fact that policosanol has different alcohols profiles which could result in different functionalities. Our future research will include the evaluation of individual alcohols contained within policosanol to identify the most bioactive alcohol. For the generation of consistent results, bioactivity-based standardization of the policosanol alcohol profile is essential.

Epidemiological studies suggest that policosanol supplementation can reduce the blood cholesterol levels in human clinical studies (26,27). Activation of AMP-kinase plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats (13,14). The principal AMP-kinase, liver kinase B1 (LKB1) phosphorylation was increased 3-fold in hepatoma cells by policosanol, slightly less than that seen with metformin, a known activator of LKB1 (9). In this study, glucose levels in blood were significantly decreased in the policosanol treatment group ($p<0.01$) similarly to the lovastatin treatment group ($p<0.001$) (Table 1 and Fig. 3). These results indicate that it is possible to manage both blood glucose and cholesterol levels using policosanol via an AMP-kinase activation process. Although a large part of this effect is attributed to the ability of policosanol to increase AMP-kinase phosphorylation and inhibit HMG-CoA reductase, the indirect potential of policosanol on blood glucose lowering cannot be excluded and is an interesting idea for further studies.

Policosanol and/or policosanol metabolites may inhibit cholesterol absorption across intestinal cells by partially oxidizing cholesterol ester fatty acids (28) or policosanol-increased bile acid excretion as shown previously (29). This will lower the blood cholesterol level by making the liver utilize cholesterol to synthesize more bile. Although we did not measure lipid content or bile acids in fecal matter, we found oily fecal matter in the rat cage changing pads during the study. Therefore, based on this possibility, fecal analysis should be performed in our follow up study, including measurement of cholesterol, bile acids, and composition etc.

We believe that this manuscript provides a preliminary rationale for how policosanol might inhibit enzymes in the pathway of cholesterol and glucose metabolism. In this study, we report that policosanol can control blood cholesterol, blood glucose, total cholesterol, triglyceride, LDL-cholesterol, and total body weight in a Wistar rat model in a similar manner to lovastatin. Our findings suggest rationale for the deeper evaluation of policosanol and the determination of the most bioactive alcohols for the observed bioactivity.

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Disclosure The authors declare no conflict of interest.

References

1. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Després JP, Fullerton HJ, Howard VJ, Huffman MD, Judd SE, Kissela BM, Lackland DT, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Matchar DB, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Willey JZ, Woo D, Yeh RW, Turner MB; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics-2015 update: A report from the American heart association. Circulation 131: e29-e322 (2014)
2. Lioyd-Jones DM, Hong Y, Labarthe D, Mozaffarian D, Appel LJ, Van Horn L, Greenlund K, Daniels S, Nichol G, Tomaselli GF, Arnett DK, Fonarow GC, Ho PM, Lauer MS, Masoudi FA, Robertson RM, Roger V, Schwamm LH, Sorlie P, Yancy CW, Rosamond WD. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. Circulation 121: 568-613 (2010)
3. Grundy SM. HMG-CoA reductase inhibitors for the treatment of hypercholesterolemia. New Engl. J. Med. 319: 24-32 (1988)
4. Endo A. The discovery and development of HMG-CoA reductase inhibitors. J. Lipid Res. 33: 1569-1582 (1992)
5. Hernandez F, Illnait J, Mas R, Castano G, Fernandez L, Gonzalez M, Cordovi N, Fernandez JC. Effects of policosanol on serum lipids and lipoproteins in healthy volunteers. Curr. Ther. Res. 51: 568-573 (1992)
6. Menedez R, Arruzarabala L, Mas R, Del Rio A, Amor A, Gonzalez RM, Carbajal D, Fraga V, Molina V, Illnait J. Cholesterol-lowering effect of policosanol on rabbits with hypercholesterolaemia induced by a wheat starch-casein diet. Brit. J. Nutr. 77: 923-932 (1997)
7. Yanai H, Katsuyama H, Hamasaki H, Abe S, Tada N, Sako A. Effects of dietary fat intake on HDL metabolism. J. Clin. Med. Res. 7: 145-149 (2015)

8. Banerjee S, Ghoshal S, Porter TD. Activation of AMP-kinase by policosanol requires peroxisomal metabolism. Lipids 46: 311-321 (2011)
9. Singh DK, Poster TD. Policosanol inhibits cholesterol synthesis in hepatoma cells by activation of AMP-kinase. J. Pharmacol. Exp. Ther. 318: 1020-1026 (2006)
10. Beg ZH, Stonik JA, Brewer HB. 3-Hydroxy-3-methyl-glutaryl coenzyme A reductase: Regulation of enzymatic activity by phosphorylation and dephosphorylation. P. Natl. Acad. Sci. USA 75: 3678-3682 (1978)
11. Menendez R, Amor AM, Conzalez RM, Fraga V, Mas R. Effect of policosanol on the hepatic cholesterol biosynthesis of normocholesterolemic rats. Biol. Res. 29: 253-257 (1996)
12. Oliaro-Bosso S, Calcio E, Mantegna S, Giraudo E, Meda C, Viola F, Cravotto G. Regulation of HMG-CoA reductase activity by policosanol and octacosadienol, a new synthetic analogue of octacosanol. Lipids 44: 907-916 (2009)
13. Shaw RJ, Lamia KA, Vasquez D, Koo SH, Bardeesy N, Depinho RA, Montminy M, Cantley LC. The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. Science 310: 1642-1646 (2005)
14. Towler MC, Hardie DG. AMP-activated protein kinase in metabolic control and insulin signaling. Circ. Res. 100: 328–341 (2007)
15. Banerjee S, Ghoshal S, Porter DT. Phosphorylation of hepatic AMP-activated protein kinase and liver kinase B1 is increased after a single oral dose of green tea extract to mice. Nutr. Res. 32: 985-990 (2012)
16. Xie Z, Dong R, Scholz D, Neumann M, Zou H. Phosphorylation of LKB1 at serine 428 by protein kinase C-zeta is required for metformin-enhanced activation of the AMP-activated protein kinase in endothelial cells. Circulation 117: 952-962 (2008)
17. Zatara G, Bar-Tana J, Kalderon B, Suter M, Morad E, Samovski D, Neumann D, Hertz R. AMPK activation by long chain fatty acyl analogs. Biochem. Pharmacol. 76: 1263-1275 (2008)
18. Renu A, Shrewsbury AM. 3-Hydroxy-3-methylglutaryl Coenzyme A reductase from rat liver. J. Biol. Chem. 251: 3815-3822 (1976)
19. Rodríguez-Echenique C, Mesa R, Más, R, Noa M, Menéndez R, González RM, Amor AM, Fraga V, Sotolongo V, Laguna A. Effect of policosanol chronically administered in male monkeys (*Macaca arctoides*). Food Chem. Toxicol. 32: 565-575 (1994)
20. Castano G, Fernadez L, Mas R, Iiinait J, Mesa M, Fernandez JC. Comparison of the effects of Policosanol and atorvastatin on lipid profile and platelet aggregation in patients with dyslipidaemia and type 2 diabetes mellitus. Clin. Drug Invest. 23: 639-650 (2003)
21. Castaño G, Mas R, Fernández L, Illnait J, Gámez R, Alvarez E. Effects of Policosanol 20 versus 40 mg/day in the treatment of patients with type II hypercholesterolemia: A 6-month double-blind study. Int. J. Clin. Pharm. Res. 21: 43-57 (2001)
22. Gouni-Berthold I, Berthold HK. Policosanol: Clinical pharmacology and therapeutic significance of a new lipid-lowering agent. Am. Heart J. 143: 356-365 (2002)
23. Heiner KB, Susanne U, Ralf D, Michael BD, Ioanna GB. Effect of policosanol on lipid levels among patients with hypercholesterolemia or combined hyperlipidemia. JAMA-J. Am. Med. Assoc. 295: 2262-2269 (2006)
24. Pons P, Rodriguez M, Robaina C, Illnait J, Mas R, Fernandez L, Fernandez JC. Effects of successive dose increases of policosanol on the lipid profile of patients with type II hypercholesterolemia and tolerability to treatment. Int. J. Clin. Pharm. Res. 14: 27-33 (1994)
25. Chen JT, Wesley R, Shamburek RD, Pucino FC. GMeta-analysis of natural therapies for hyperlipidemia: Plant sterols and stanols versus policosanol. Pharmacotherapy 25: 171-183 (2005)
26. Barrat E, Zaïr Y, Sirvent P, Chauveau P, Maudet C, Housez B, Derbord E, Lescuyer JF, Bard JM, Cazaubiel M, Peltier SL. Effect on LDL-cholesterol of a large dose of a dietary supplement with plant extracts in subjects with untreated moderate hypercholesterolaemia: A randomised, double-blind, placebo-controlled study. Eur. J. Nutr. 52: 1843-1852 (2013)
27. Ogier N, Amiot MJ, Georgé S, Maillot M, Mallmann C, Maraninchi M, Morange S, Lescuyer JF, Peltier SL, Cardinault N. LDL-cholesterol-lowering effect of a dietary supplement with plant extracts in subjects with moderate hypercholesterolemia. Eur. J. Nutr. 52: 547-557 (2013)
28. Li Q, Thomson AB, Clandinin MT. Cholesterol ester and free fatty acids are modulated by policosanol in CaCo-2 intestinal cell. J. Am. Coll. Nutr. 30: 201-209 (2011)
29. Chi HN, Ka YL, Yu H, Zhen YC. Policosanol has no antioxidant activity in human low-density lipoprotein but increase excretion of bile acids in hamsters. J. Agr. Food Chem. 53: 6289-6293 (2005)