Case Report

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Internal Medicine Section

Improvement in Lipid Profile after Long-term Consumption of Policosanol Accompanied by Reduced Oxidation of LDL and Aortic Stiffness via CETP Inhibition in Healthy Middle-aged Women

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ABSTRACT

Cuban Policosanol (PCO) was reported to lower serum Total Cholesterol (TC) as well as increase High-Density Lipoprotein Cholesterol (HDL-C) and enhance HDL functionality. In this study, we compared changes in the blood lipid and lipoprotein profiles in hypercholesterolemic women subjects (50-year-old) who consumed policosanol for 20 weeks. At week 0, subject had high TC but low HDL-C levels. After 20 weeks of policosanol consumption, serum TC and Triglyceride (TG) levels were reduced by 10% and 44%, respectively. HDL-C level was elevated 1.7-fold while Low-Density Lipoprotein (LDL) level was reduced by up to 20%. Serum Cholesteryl Ester Transfer Protein (CETP) activity was reduced from 33% CE-transfer at week 0 to 22% CE-transfer at week 20. Glycation extent was significantly reduced in all lipoprotein fractions, especially in LDL and HDL_2. In Very Low-Density Lipoprotein (VLDL) and LDL, cholesterol and TG contents were reduced. In HDL_2 and HDL_3, cholesterol was more enriched and TG content reduced. LDL was more resistant to cupric ion-mediated oxidation and showed less atherogenic properties via phagocytosis into macrophages. Apolipoprotein A-I (apoA-I) was more enriched in HDL_2 and HDL_3 in a time-dependent manner. The antioxidant ability of HDL was enhanced by 25% in terms of ferric ion reduction ability and paraoxonase activity. In conclusion, 20 weeks of policosanol consumption improved the lipid profile by increasing HDL-C, and lipoprotein functionality to enhance antioxidant, anti-glycation, and anti-atherogenic properties via CETP inhibition.

Keywords: Glycation, Hypercholesterolemia, Lipid profile, Lipoproteins

CASE REPORT

A 50-year-old female with hypercholesterolemia consumed policosanol for 20 weeks. She was an office worker for 22 years at Yeungnam University. She had taken daily 10 mg of policosanol (Rainbow & Nature Pvt., Ltd., Thornleigh, NSW, Australia) at the same time every evening with meals around 7-9 pm and consumed a typical Korean diet, which is enriched with rice and vegetables, and no changes in dietary patterns were observed in the past five years. The subject had maintained a sedentary lifestyle without exercise for the last five years. Before the study, the subject had suffered from cold hands and feet almost every night. Here, we investigated a case report of a hypercholesterolemic Korean middle-aged women (age) with low HDL-C who was treated with policosanol for 20 weeks to monitor the treatment efficacy in terms of lipid and lipoprotein functionality. Furthermore, blood pressure was monitored at four-week intervals for 20 weeks. Earlier the doctor had prescribed her to statin due to mild hypercholesterolemia but she quitted three months before policosanol consumption. No other medical record and family history was known.

Blood pressure was measured each visit for a total of three times, and the average was recorded at four-week intervals. Blood was obtained from the subject each visit following overnight fasting. Blood was collected using a vacutainer (BD Biosciences, Franklin Lakes, NJ, USA) containing EDTA (final concentration of 1 mM). TC, TG, HDL-C, and glucose were measured using commercially available kits (Cleantech TS-S; Wako Pure Chemical, Osaka, Japan). The Ferric Reducing Ability of Plasma (FRAP) was determined using the method described by Benzie and Strain [1]. The antioxidant activities of HDL fractions (20 μg each in PBS) were estimated by measuring increases in absorbance induced by generated ferrous ions. Paraoxonase-1 (PON-1) activity was determined by measuring the initial velocity of p-nitrophenol production at 37°C based on its absorbance at 405 nm (Microplate reader, Bio-Rad model 680; Bio-Rad, Hercules, CA, USA), as described [2]. Very Low-Density Lipoprotein (VLDL, d <1.019 g/mL), Low-Density Lipoprotein (LDL, 1.019 <d <1.063), High-Density $Lipoprotein_2$ (HDL_2, 1.063 <d <1.125), and High-Density $Lipoprotein_3$ (HDL_3, 1.125 <d <1.225) were isolated from the individual plasma of each group via sequential ultracentrifugation [3].

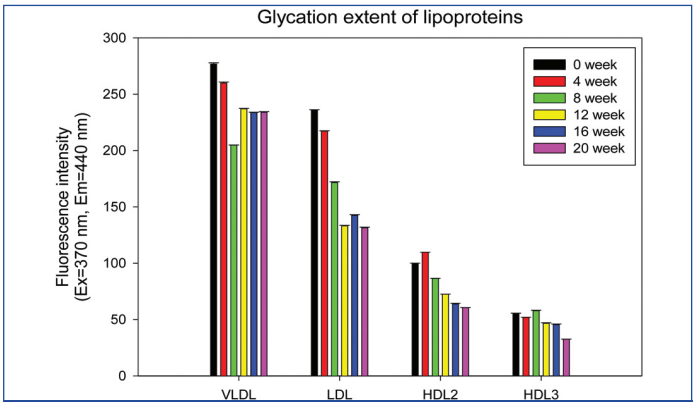
To differentiate the extent of glycation in the lipoproteins, it was determined by reading fluorometric intensities at 370 nm (excitation) and 440 nm (emission), as described [4] using a spectrofluorometer LS55 (Perkin-Elmer, Shelton, CT, USA).

Cholesteryl ester transfer protein assay was carried out according to our previous report [5]. Oxidized LDL (oxLDL) was obtained by incubation of the LDL fraction with $CuSO_4$ (final concentration of 10 μM) for four hours at 37°C. The comparison of electromobility of LDL between weeks 0, 4, 8, 12, 16 and 20 with or without cupric ion in 0.5% agarose gel were measured [Table/Fig-1]. The extents of oxidized species in LDL and cell culture media were measured by thiobarbituric acid reactive substances (TBARS) assay [6]. Phagocytosis of LDL into macrophages was carried out in THP-1 cells, a human monocytic cell line, which were incubated in medium containing phorbol 12-myristate 13-acetate (PMA, 150 nM) in 24-well plates for 48 hours at 37°C in a humidified incubator (5% CO_2, 95% air) in order to induce differentiation into macrophages. Cells were stained with oil-red O staining solution (0.67%) and washed with distilled water. THP-1 macrophage-derived foam cells were then observed and photographed using a Nikon Eclipse TE2000 microscope (Tokyo, Japan) at 400x magnification, as in our previous report [6,7]. After 20 weeks of policosanol consumption, glycation

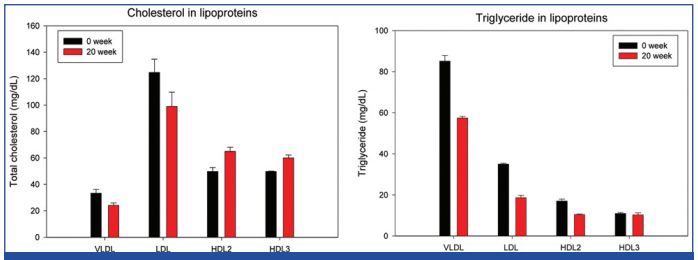
extent was significantly reduced in all lipoprotein fractions (VLDL, LDL, HDL_2, and HDL_3) Especially, LDL and HDL_2 showed 45% and 40% reduction of glycation, respectively, at week 20 under the same protein content [Table/Fig-2]. [Table/Fig-3] displays the cholesterol and triglyceride contents of various lipoprotein fractions (VLDL, LDL, HDL_2, and HDL_3). Comparison of electromobility of native LDL or oxidized LDL in the presence of cupric ion during policosanol consumption is shown in [Table/Fig-4]. All LDL during policosanol consumption showed less mobility than LDL from week 0, except at 16 weeks. Clearer band intensity and slower electromobility indicate that LDL was less oxidized, and policosanol consumption protected LDL from oxidation. Quantification of oxidized species in cell culture media showed that LDL from week 0 resulted in a 10-fold higher level of MDA (around 4.5 μM) than PBS in media [Table/Fig-5]. However, LDL from week 20 resulted in a 54% lower MDA level (around 1.4 μM) in media. Agarose electrophoresis revealed that band intensities of HDL_2 and HDL_3 increased in a time-dependent manner [Table/Fig-6]. SDS-PAGE also revealed that increased apoA-I band intensities in HDL_2 and HDL_3 with policosanol consumption [Table/Fig-6]. At week 20, HDL_2 showed 25% more enhanced FRAP ability than that at week 0 [Table/Fig-7a]. PON activity was enhanced 1.6-fold by policosanol consumption (week 20), as shown in [Table/Fig-7b].

Parameters	Week 0	Week 4	Week 8	Week 12	Week 16	Week 20
BMI (kg/m^2)	17.8	17.7	18	17.6	18.2	17.9
Body fat (kg)	7.3	7.8	7.3	7.7	7.2	7.4
Body moisture (AU)	29.8	29.3	30	28.7	30.2	29.7
Muscle mass (kg)	38.5	37.9	38.8	37.1	39.1	38.4
Visceral fat (kg)	0.6	0.6	0.6	0.6	0.6	0.6
Subcutaneous fat (kg)	6.7	7.2	6.7	7.1	6.6	6.8
SBP (mmHg)	110	113	109	115	118	126
DBP (mmHg)	70	69	65	68	79	80
Heart rate (BPM)	62	65	69	65	62	65
TC (mg/dL)	238	245	240	235	228	216
TG (mg/dL)	99	54	38	46	75	56
HDL-C (mg/dL)	42	51	56	55	66	72
% HDL-C in TC	17	20	23	22	29	32
TG/HDL-C	2.4	1.1	0.7	0.8	1.1	0.7
LDL-C (mg/dL)	176	183	176	179	147	141
CETP activity (CE-transfer, %)	33	30	25	26	23	22
Glucose (mg/dL)	81	89	91	82	94	82

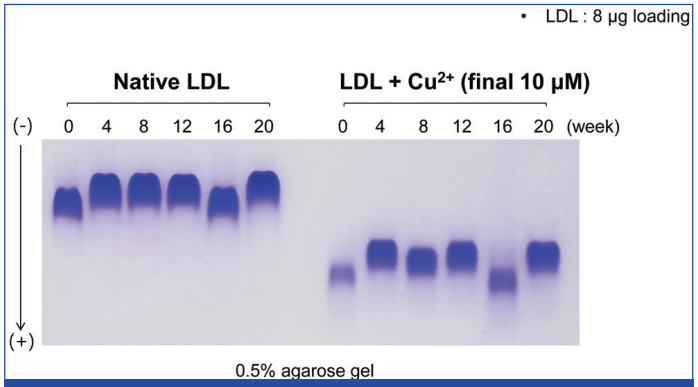
[Table/Fig-1]: Anthropometric parameters and biochemical analysis after 20 weeks of policosanol consumption.
BMI: Body mass index; AU: Arbitrary units; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BPM: Beats per minutes; TC=Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; CETP: Cholesteryl ester transfer protein



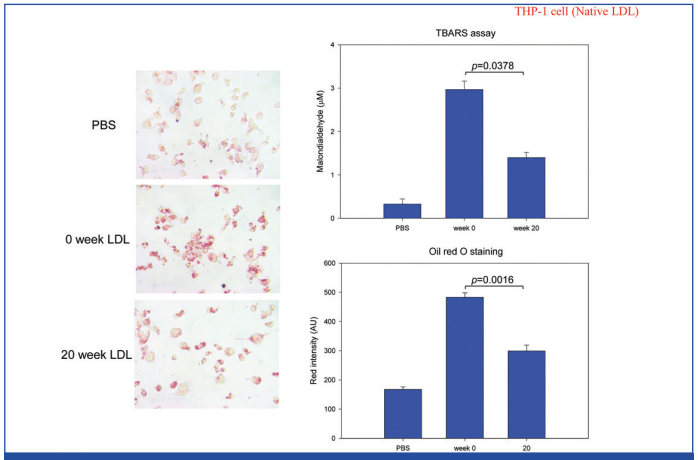
[Table/Fig-2]: Fluorometric determination of glycation extent of lipoproteins during policosanol consumption. VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL_2 & HDL_3, high density lipoprotein subfractions.



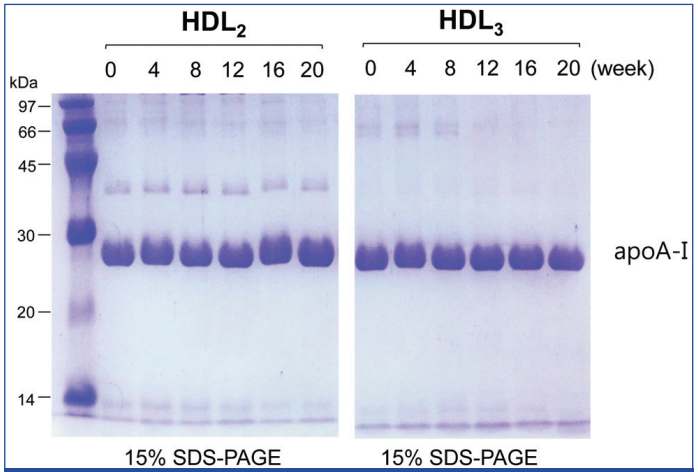
[Table/Fig-3]: Cholesterol and triglyceride contents in various lipoproteins (VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL2 & HDL3, high density lipoprotein subfractions).



[Table/Fig-4]: Comparison of electromobility of native LDL or oxidized LDL under the presence of cupric ion during policosanol consumption.



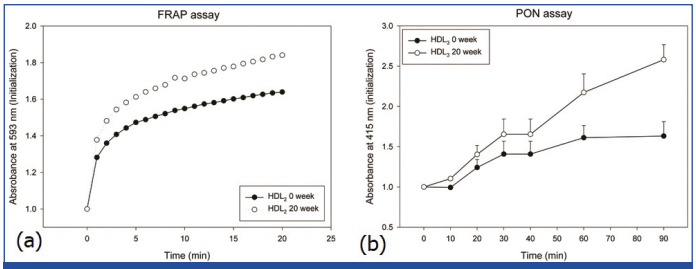
[Table/Fig-5]: Uptake of LDL into macrophages and extent of oxidized species during policosanol consumption (quantification of oxidized species in cell culture media using the TBARS method, quantification of oil-red O-stained area by computer-assisted morphometry).



[Table/Fig-6]: Electrophoretic patterns of HDL_2 and HDL_3 during policosanol consumption at weeks 0,4,8,12,16 and 20 (5 μg/lane, 15% SDS-PAGE).

DISCUSSION

Policosanol might contribute to longevity via CETP inhibition [Table/Fig-1], elevation of HDL-C, and enhanced functionality of HDL, as in our previous report [8,9]. Low HDL levels (<40 mg/dL)



[Table/Fig-7]: (a) Antioxidant ability (ferric ion reduction ability of HDL_2 from week 0 and week 20). (b) Paraoxonase activity of HDL_2 and HDL_3 during policosanol consumption.

are considered as an independent risk factor for CHD [10]. This current case report in one hypercholesterolemic female reveals that consumption of policosanol reduced the risk of glycation in all lipoproteins in a time-dependent manner [Table/Fig-2]. The triglyceride contents in lipoprotein fractions (VLDL, LDL, HDL_2, and HDL_3) were reduced after 20 weeks of policosanol consumption, and the cholesterol content was higher in good cholesterol (HDL_2 and HDL_3). Moreover, consumption of policosanol inhibited cupric ion-mediated oxidation of LDL and reduced cell-mediated (human monocytic cell line) generation of Malondialdehyde (MDA) by LDL. Although the female subject had higher cholesterol and lower HDL-C than normal, she showed normal ranges of blood pressure and body fat contents. TC gradually and slowly decreased while TG more rapidly decreased with a gradual decrease in CETP activity. The subject showed an almost 2-fold increase in %HDL-C in TC at week 0 and week 20, 17%, and 32%, respectively.

In the same context, apoA-I was elevated in HDL with enhanced antioxidant activity such as FRAP and paraoxonase activity. Moreover, enhanced antioxidant activity was connected to reduced oxidation of LDL [Table/Fig-4] and less uptake of LDL at week 20 [Table/Fig-5] since PON activity in HDL is involved in the removal of oxidized species in LDL. Higher apoA-I content is associated with stronger PON activity, which exerts anti-inflammatory activity against oxidation of LDL [11]. Elevated CETP activity is associated with increased serum TG and TG-enriched LDL levels. It is well established that serum TG level is an independent risk factor of inflammatory disease. TG levels are an important and independent predictor of Coronary Artery Disease (CAD) and stroke in the Asia-Pacific region [12]. In the current study, policosanol consumption caused a remarkable decrease in serum TG and increase in HDL-C via CETP inhibition accompanied by inhibition of LDL oxidation and phagocytosis into macrophages.

CONCLUSION

In a middle-aged woman, 20 weeks of policosanol consumption improved the lipid profile, including lowering of TC and TG and elevation of HDL-C. The pain of cold hands and feet every night disappeared after policosanol consumption. Lipoprotein functionality was also enhanced along with less oxidation and glycation, resulting in improved anti-atherogenic properties and vascular functions. These results suggest that Cuban policosanol can induce beneficial effects to suppress cardiometabolic risk.

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REFERENCES

[1] Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power : The FRAP assay. Anal Biochem. 1996;239(1):70-76.
[2] Park KH, Shin DG, Kim JR, Hong JH, Cho KH. The functional and compositional properties of lipoproteins are altered in patients with metabolic syndrome with increased cholesteryl ester transfer protein activity. Int J Mol Med. 2010;25(1):129-36.
[3] Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest. 1955;34(9):1345-53.
[4] McPherson JD, Shilton BH, Walton DJ. Role of fructose in glycation and cross-linking of proteins. Biochemistry. 1988;27(6):1901-07.
[5] Cho K-H. Synthesis of reconstituted high density lipoprotein (rHDL) containing apoA-I and apoC-III: the functional role of apoC-III in rHDL. Mol Cells. 2009;27(3):291-97.
[6] Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617):1199-200.
[7] Park KH, Jang W, Kim KY, Kim JR, Cho KH. Fructated apolipoprotein A-I showed severe structural modification and loss of beneficial functions in lipid-free and lipid-bound state with acceleration of atherosclerosis and senescence. Biochem Biophys Res Commun. 2010;392(3):295-300.
[8] Lee EY, Yoo JA, Lim SM, Cho KH. Anti-aging and tissue regeneration ability of policosanol along with lipid-lowering effect in hyperlipidemic zebrafish via enhancement of high-density lipoprotein functionality. Rejuvenation Res. 2016;19(2):149-58.
[9] Kim JY, Kim SM, Kim SJ, Lee EY, Kim JR, Cho KH. Consumption of policosanol enhances HDL functionality via CETP inhibition and reduces blood pressure and visceral fat in young and middle-aged subjects. Int J Mol Med. 2017;39(4):889-99.
[10] Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation. 1989;79(1):8-15.
[11] James RW, Deakin SP. The contribution of high density lipoprotein apolipoproteins and derivatives to serum paraoxonase-1 activity and function. Adv Exp Med Biol. 2010;660:173-81.
[12] Patel A, Barzi F, Jamrozik K, Lam TH, Ueshima H, Whitlock G, et al. Serum triglycerides as a risk factor for cardiovascular diseases in the Asia-Pacific region. Circulation. 2004;110(1):2678-86.

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